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/BIX is also provided which comprises both /BI and /ABEX <<<

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=> d all abeq tech abex tot l116

L116 ANSWER 1 OF 5 WPIX COPYRIGHT 2003 THOMSON DERWENT on STN
AN **2003-148342** [14] WPIX
DNC **C2003-038281**
TI Synthesis of proteins using cell-free protein system, e.g. membrane proteins, receptors, or channel proteins, useful for developing drugs, comprises adding surfactant to the system to produce the protein without agglutination.
DC B04 D16
IN ISHIHARA, G; **KIGAWA, T**; SHIROUZU, M; TAJIMA, K; YABUKI, T; **YOKOYAMA, S**
PA (RIKE) RIKEN KK
CYC 22
PI WO 2002090537 A1 20021114 (200314)* JA 45p C12N015-09 <--
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: CA US
JP 2003018999 A 20030121 (200317) 15p C12P021-02 <--
ADT WO 2002090537 A1 WO 2002-JP4204 20020426; JP 2003018999 A JP 2001-285145 20010919
PRAI JP 2001-285145 20010919; JP 2001-135111 20010502
IC ICM **C12P021-02**
ICS **C07K001-02**; **C12N001-21**
ICA **C12N015-09**
AB WO 200290537 A UPAB: 20030227
NOVELTY - Synthesis (M1) of a protein by using a cell-free protein synthesis system comprises adding a surfactant to the system so that the protein can be produced without agglutination.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) A similar method (M2) in which an extractant for bacterial mass is applied as well;
(2) Restructuring (M3) a protein produced in a cell-free protein synthesis system containing a template nucleic acid encoding at least a part of a membrane protein, surfactant and lipid, in which and concentration of the surfactant in the synthetic reaction solution is

lowered during the protein synthesis or a definite period of time afterwards; and

(3) Synthesizing (M4) a membrane protein that has at least a part of its biological activity comprising:

(a) synthesis of the protein in a cell-free protein synthesis system containing a cell extraction solution, a template nucleic acid encoding at least a part of a membrane protein, surfactant and lipid; and

(b) lowering concentration of the surfactant in the synthetic reaction solution during the protein synthesis or after a definite period of time.

USE - The method is useful for synthesis of proteins, restructuring proteins, and synthesizing a membrane protein (claimed), especially proteins with hydrophobic domains e.g. membrane proteins, receptors, channel proteins or transporters, which are then applicable in studying their structure and function and in developing drugs to control such functions and in diagnosis and treatment of related diseases.

ADVANTAGE - Such method is efficient and quantitative, without loss of protein activity due to problems in agglutination of difficult to dissolve proteins and in the folding process, on a large scale.

Dwg.0/11

FS CPI

FA AB; DCN

MC CPI: B01-A03; B04-C03C; B04-E01; B04-E03F; B04-K0100E; B04-L0100E; B04-N0400E; B05-B01P; B07-A02B; B10-A22; B10-D03; B10-E04C; B11-C08; B12-K04A; B12-K04E; D05-H09; D05-H12A; D05-H17

TECH UPTX: 20030227

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Processes: The protein is a protein containing at least a hydrophobic domain, which is particularly a membrane protein, including receptor, channel protein, transporter and membrane-binding enzyme, or their fragment.

TECHNOLOGY FOCUS - ORGANIC CHEMISTRY - Preferred Processes: Such surfactant is a mild surfactant which will not denature the protein, e.g. a non-ionic or amphoteric surfactant including digotonin, polyoxyethylene alkyl ether, polyoxyethylene sorbitan, beta-dodecyl maltoside, beta-octyl glucoside, beta-nonyl glucoside, beta-heptyl thioglucoside, beta-octyl thioglucoside, sucrose monodecanoate, sucrose monodecanoate, octyltetraoxyethylene, octylpentaoxyethylene, dodecyloctaoxyethylene, N,N-dimethyldecylamine N-oxide, N,N-dimethyldodecylamine N-oxide, N,N-dimethyldodecylammoniopropanesulfonate, octyl(hyeroxyethyl) sulfoxide, octanoyl-N-methylglucamide, nonanoyl-N-methylglucamide, decanoyl-N-methylglucamide and 3-((3-cholamidopropyl)-dimethylammonio)-1-propanesulfonate (CHAPS). Concentration of such surfactant is particularly 0.1-2 volume % digotonin and/or 0.01-0.5 volume % Brij 35. To lower the surfactant concentration, dialysis, dilution, filtration, centrifugation and/or addition of an adsorbent for adsorption of such surfactant is applied.

ABEX UPTX: 20030227

EXAMPLE - To synthesize a rat neurotensin receptor (NRT), an MBP-T43NTR-TrxA-H10 template fragment was prepared after two PCR reactions, with Escherichia coli S30 extract (Zubay et al., Annu. Rev. Geneti., 1973, vol. 7, p. 267) for reaction in a cell-free protein synthesis system with e.g. 0.4% digotonin added. The required protein was easily recovered.

L116 ANSWER 2 OF 5 WPIX COPYRIGHT 2003 THOMSON DERWENT on STN

AN **2003-140656** [13] WPIX

DNN **N2003-111659** DNC **C2003-035853**

TI Structural analysis of proteins useful for identifying proteins, their domain structures and global stereostructures, comprises synthesis of protein with tag sequence and measuring by mass spectrometry.

DC B04 D16 S03

IN **KIGAWA, T**; SHEN, X; **YOKOYAMA, S**

PA (RIKA) RIKAGAKU KENKYUSHO; (RIKE) RIKEN KK
CYC 22
PI WO 2002099437 A1 20021212 (200313)* JA 34p G01N033-68 <--
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: CA US
JP 2002372517 A 20021226 (200314) 13p G01N027-62 <--
ADT WO 2002099437 A1 WO 2002-JP4473 20020508; JP 2002372517 A JP 2001-164819 20010531
PRAI JP 2001-164819 20010531
IC ICM **G01N027-62; G01N033-68**
ICS **C12N015-09; C12Q001-37; G01N033-483; G01N033-53**
AB WO 200299437 A UPAB: 20030224
NOVELTY - Identifying the molecular weight of a protein by mass spectrometry comprises:
(a) synthesizing a protein fused with a tag sequence, in a cell-free protein synthesis system;
(b) contacting the fused protein with a tag sequence-binding carrier;
(c) collecting the bonded carrier; and
(d) measuring molecular weight of the fused protein thus collected.
DETAILED DESCRIPTION - Identifying (M1) the molecular weight of a protein by mass spectrometry (MS) comprises:
(a) synthesizing a protein fused with a tag sequence in a cell-free protein synthesis system;
(b) contacting the fused protein with a carrier capable of binding to the tag sequence;
(c) collecting the carrier bonded to the fused protein; and
(d) measuring molecular weight of the fused protein by MS with use of the fused protein bonded to the carrier as collected above.
INDEPENDENT CLAIMS are also included for the following:
(1) Identifying (M2) a protein domain by MS comprising:
(a) synthesizing a protein fused with a tag sequence in a cell-free protein synthesis system;
(b) contacting the fused protein with a carrier capable of binding to the tag sequence;
(c) collecting the carrier bonded to the fused protein;
(d) partial decomposition of the fused protein bonded to the carrier as collected above by protease; and
(e) measuring molecular weight of the fused protein and/or decomposition products by MS with use of solution of the decomposition reaction; and
(2) Identifying (M3) a protein domain by MS comprising:
(a) synthesizing a protein fused with a tag sequence in a cell-free protein synthesis system;
(b) contacting the fused protein with a carrier capable of binding to the tag sequence;
(c) collecting the carrier bonded to the fused protein;
(d) measuring molecular weight of the fused protein by MS with use of the fused protein bonded to the carrier as collected above;
(e) partial decomposition of the fused protein bonded to the carrier as collected by protease, and measuring molecular weight of the decomposition product by MS with use of solution of the decomposition reaction; and
(f) comparing such measured molecular weight of the fused protein and that of the decomposition product.
USE - The analytical method is applicable in identifying the molecular weight of proteins (claimed), their domain structures and studying other global stereostructures, and structural genomic investigation.
ADVANTAGE - With such method, synthesis, purification and analysis of a protein can be efficiently and quickly carried out, particularly on multi-specimen screening plates consecutively with automation, in a high-throughput manner as stably adsorbed entity, without problems due to

the insoluble purified protein.
Dwg.0/4
FS CPI EPI
FA AB; DCN
MC CPI: B04-B03C; B04-C01; B04-G01; B04-K01; B04-N0400E; B11-C08A; B11-C08D; B11-C08E3; B11-C08F2; B12-K04E; D05-A02C; D05-H09; D05-H11; D05-H17
EPI: S03-E10; S03-E14H; S03-E14H4
TECH UPTX: 20030224
TECHNOLOGY FOCUS - ORGANIC CHEMISTRY - Preferred Method: The carrier capable of binding specifically to the tag sequence is a carrier holding 1 or more selected from antibodies, binding proteins, receptors and parts containing their binding regions; or chosen from metal atoms, amino acids, peptides, oligonucleotides and heterocyclic compounds. The MS can be time-of-flight type matrix-assisted laser-desorption ionization technique (MALDI-TOF).
ABEX UPTX: 20030224
EXAMPLE - Identifying the molecular weight of a protein by mass spectrometry was carried out as follows. A His-Ras protein was synthesized in a cell-free protein synthesis system (Annu. Rev. Genet., 1973, vol. 7, p. 267, then purified on nickel-bound agarose-beads and MALDI-TOF MS analysis of the agarose bead-adsorbed Ras protein, with observation of (Ras)+ peak at m/z = 22108.1 and (Ras)2+ peak at m/z 11053.5.

L116 ANSWER 3 OF 5 WPIX COPYRIGHT 2003 THOMSON DERWENT on STN
AN **2003-138616** [13] WPIX
DNC **C2003-035267**
TI Producing a soluble protein domain comprises expressing a fusion protein that is coded on each of the DNA fragments fused with a DNA encoding a functional protein and synthesizing the soluble protein domain in a **cell-free** system.
DC B04 D16
IN **KIGAWA, T; SEKI, E; YOKOYAMA, S**
PA (RIKE) RIKEN KK; (KIGA-I) KIGAWA T; (SEKI-I) SEKI E; (YOKO-I) YOKOYAMA S
CYC 3
PI US 2002142387 A1 20021003 (200313)* 12p C12P021-02 <--
CA 2362784 A1 20020906 (200313) EN C12N015-11 <--
JP 2002262873 A 20020917 (200313) 9p C12N015-09 <--
ADT US 2002142387 A1 US 2001-994573 20011126; CA 2362784 A1 CA 2001-2362784 20011123; JP 2002262873 A **JP 2001-62703 20010306**
PRAI **JP 2001-62703 20010306**
IC ICM **C12N015-09; C12N015-11; C12P021-02**
ICS **C07K014-00; C07K014-47; C07K019-00; C12N001-21; C12N015-62; C12N015-70; C12P019-34; C12P021-00; C12Q001-68; G01N023-20; G01N024-08; G01N033-566; G01N033-68**
ICI **C12P021-02**; C12R001:19
AB US2002142387 A UPAB: 20030224
NOVELTY - Producing a soluble protein domain comprising preparing two or more DNA fragments by partially digesting a DNA coding for a protein, expressing a fusion protein that is coded on each of the DNA fragments fused with a DNA encoding a functional protein exhibiting a function, selecting the fusion protein exhibiting the function, and synthesizing the soluble protein domain in a **cell-free** system, is new.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method for analyzing the three dimensional structure of a protein.
USE - The method is useful for producing a soluble protein domain (claimed).
Dwg.0/5
FS CPI
FA AB; DCN
MC CPI: B04-N0400E; B04-N08; B11-C08G1; B11-C08G2; B12-K04E; D05-H09; D05-H17C

TECH UPTX: 20030224
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: Producing a soluble protein domain further comprises:
(a) selecting a fusion protein from the fusion proteins containing a functional protein exhibiting a function; and
(b) synthesizing a soluble protein domain from the fusion protein selected from step (a).
The method also comprises:
(a) constructing an expression vector that expresses a fusion protein with a **green fluorescent** protein or its derivative, where
the expression vector comprises a DNA coding for a protein and a gene for the **green fluorescent** protein or its derivative;
(b) preparing two or more DNA fragments by partially digesting the expression vector with DNA decomposition enzyme;
(c) transforming Escherichia coli with each of the DNA fragments to obtain two or more transformed E. coli isolating a transformed clone that emits fluorescence among the transformed E. coli;
(d) recovering the DNA from the isolated transformed clone; and
(e) synthesizing the soluble protein domain that is coded on the recovered DNA in a **cell-free** system.
The method may also comprise:
(a) preparing an expression vector comprising a DNA coding for a protein and a DNA coding for a functional protein;
(b) treating the vector by using a decomposing enzyme and forming two or more vectors, each vector comprising a fragment of the DNA coding for a protein and the DNA coding for a functional protein;
(c) expressing a fusion protein that is coded on each of the vectors fused with a DNA encoding a functional protein;
(d) selecting the fusion protein exhibiting the function among two or more fusion proteins synthesized in step (a);
(e) synthesizing the soluble protein domain in a **cell-free** system.
The functional protein consists of enzyme, binding protein, luminescent protein, fluorescent protein or its functional protein. The fluorescent protein is a **green fluorescent** protein or its
derivative. The selection is performed by:
(a) transforming a recipient **cell** with each of the DNA fragments and the DNA of the functional protein; and
(b) selecting the clone that exhibits the function in the obtained transformants.
The selection may also be performed by measuring the function of the expressed proteins. The recipient **cell** is E. coli. Analyzing the three dimensional structure of a protein comprises:
(a) synthesizing the soluble protein domain by the method of producing a soluble protein domain; and
(b) analyzing the three dimensional structure of the soluble protein domain by X-ray crystallography or NMR spectroscopy.

L116 ANSWER 4 OF 5 WPIX COPYRIGHT 2003 THOMSON DERWENT on STN
AN **2001-257972** [26] WPIX
DNC **C2001-077772**
TI New isolated, enriched, **cell free** and/or recombinant nucleic acid useful for e.g. altering **cell** proliferation characteristic such as to alter plant **cell**, organ or tissue size.
DC C06 D16
IN GUTIERREZ-ARMENTA, C; RAMIREZ-PARRA, E
PA (CNSJ) CONSEJO SUPERIOR INVESTIGACIONES CIENTIF
CYC 93
PI WO 2001021644 A2 20010329 (200126)* EN 77p C07K014-00 <--
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM EE

ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR
LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI
SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000077814 A 20010424 (200141) C07K014-00 <--
EP 1214436 A2 20020619 (200240) EN C12N015-82 <--
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI
BR 2000014634 A 20030225 (200320) C07K014-00 <--
JP 2003510040 W 20030318 (200321) 89p C12N015-09 <--
ES 2182615 A1 20030301 (200327) C07K014-415 <--

ADT WO 2001021644 A2 WO 2000-EP9325 20000925; AU 2000077814 A AU 2000-77814 20000925; EP 1214436 A2 EP 2000-967766 20000925, WO 2000-EP9325 20000925; BR 2000014634 A BR 2000-14634 20000925, WO 2000-EP9325 20000925; JP 2003510040 W WO 2000-EP9325 20000925, JP 2001-525218 20000925; ES 2182615 A1 ES 1999-2127 19990924

FDT AU 2000077814 A Based on WO 200121644; EP 1214436 A2 Based on WO 200121644; BR 2000014634 A Based on WO 200121644; JP 2003510040 W Based on WO 200121644

PRAI ES 1999-2474 19991111; ES 1999-2127 19990924

IC ICM **C07K014-00; C07K014-415; C12N015-09; C12N015-82**
ICS A01H005-00; **C07K016-16;** C12N005-04; C12N005-10; C12N005-14; **C12N015-11; C12N015-29; C12N015-62;** C12Q001-68

AB WO 200121644 A UPAB: 20010515
NOVELTY - An isolated, enriched, **cell free** and/or recombinant nucleic acid (II) comprising a sequence encoding for expression of a protein or peptide (I) capable of altering E2F-dimerization partner (DP) activity in a plant **cell** is new.

DETAILED DESCRIPTION - (P1) is characterized in that it has one or both DP activities in plants selected from:

(1) the ability to dimerized with plant E2F protein; and

(2) the ability to modulate, particularly enhance, E2F binding to E2F transcription factor binding sites in plant DNA or effect of it characterized in that the protein or peptide comprises a 261 amino acid sequence (S1) or a functionally active part of it or a sequence having at least 70% homology to so the sequence or part of it.

INDEPENDENT CLAIMS are also included for the following:

(1) controlling one or more of plant growth, gene expression, cellular DNA replication, cell cycle progression, differentiation and development comprising increasing or decreasing DP protein activity in a plant cell through the expression of (P1);

(2) isolated (I);

(3) a nucleic acid probe (III) comprising a DNA sequence corresponding to an amino acid sequence comprising 261, 458, 52, or 51 amino acids fully defined in the specification;

(4) a nucleic acid probe or primer (IV) comprising a double or single stranded DNA of sequence corresponding to 10 or more contiguous nucleotides taken from a sequence (S2) comprising 1089 base pairs fully defined in the specification, with the proviso that it is not selected from residues 70-136;

(5) an oligonucleotides probe (V) comprising at least 18 contiguous bases of S2;

(6) an antisense DNA (VI) to (II), (III), (IV) or (V);

(7) a nucleic acid vector or construct (VII) comprising (II), (III), (IV), (V), or (VI) or its antisense nucleic acid;

(8) a plant cell (IX) comprising (VIII);

(9) a transgenic plant or its part (X) comprising (IX);

(10) an antibody (XI) raised against (I);

(11) identifying and/or isolating (M2) DNAs corresponding to complete or partial genes that are regulated in G1 passage, G1/S phase transition and/or S phase progression of the cell cycle, involves contacting sample of genomic DNA with binding material specific for binding such complete or

partial genes, removing non-bound DNA from the specific binding material, and releasing and isolating the bound DNA, where the specific binding material comprises a peptide or protein including the DNA binding sequence of a protein that is capable of acting as a part of a plant hetero-oligomer transcription activator or repressor; and

(12) a specific binding material (XII) which comprises a peptide or protein having DNA binding activity with respect to plant DNA transcription activator or repressor factor binding sites, particularly in genomic DNA, and having the ability to dimerize or oligomerize with a further such plant protein together with one or more of a further peptide or protein.

USE - (IV) is useful in Northern and Southern blotting and in polymerase chain reaction (PCR), including RT-PCR and LCR (claimed). (M1) or (II) is useful for altering plant cell, organ or tissue shape, and particularly to alter cell proliferation characteristic such as to alter plant cell, organ or tissue size. (M1) is useful for altering expression of other proteins with transformed cells and cells derived from it, particularly direct or indirect progeny. (II) is useful for obtaining sequences of (II) and, by using computational homology analysis of other organism's genome e.g. of plant, animal, yeast, bacteria, virus or fungal genomes, or molecular biology probing or other analysis techniques to identify therapeutic or otherwise industrially useful target genes involved in cell cycle that are no readily obtainable due to the comparative complexity or lack of information of/on such systems. (XI) is useful for identification and isolation of (I) and (II).

Dwg.0/3

FS CPI

FA AB; DCN

MC CPI: C04-A08OOE; C04-C01G; C04-E03F; C04-E05; C04-E06; C04-E08; C04-F08OOE; C04-G0100E; C04-N04A0E; C11-C07A; C11-C08E5; C12-K04E; C12-K04F; D05-H09; D05-H11; D05-H12A; D05-H12D1; D05-H12D2; D05-H12E; D05-H14B3; D05-H16B

TECH UPTX: 20010515

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: The plant DP activity comprises one or both of the ability to dimerize with plant E2F protein, and the ability to modulate E2F binding to E2F/DP transcription factor binding sites in plant DNA. M1 comprises altering the plant DP protein level, the E2F-DP DNA binding activity, transactivation properties and/or the DP/E2F binding activity. DP may be modified alone and/or in combination with a modification of the levels or activity of plant E2F and/or plant Rb. In M2, the specific binding material comprises a peptide or protein which includes a plant E2F DNA binding domain, together with a plant E2F-DP hetero-dimerization domain and/or a plant retinoblastoma protein E2F binding domain. The E2F and DP domains are comprised in Triticum monococcum sequences or its functional variants or parts, or the DP domains are comprised in (II). The specific binding material peptide or protein is labeled or tagged to assist in identifying or immobilizing it, particularly when in bound complex with the gene to be identified and/or isolated, and comprises two peptides or proteins, one including the E2F DNA binding domain and one including the DP hetero-dimerization domain bound together as a hetero-dimer. The E2F sequence is all or part of a sequence comprising 458 amino acids fully defined in the specification or a sequence having 90% homology to it. Preferred Polypeptide: (I) has at least 50% or more, preferably 70% or more identity with S1, or its functional part. (I) of sequence S1, or its variant modified such that the amino acid sequence is mutated so that its ability to dimerize with E2F protein is reduced from that of the native sequence or abolished completely, where the peptide is capable of acting as a DP protein which decreases or abolishes native or recombinant E2F binding to its DNA binding site, thus inhibiting or abolishing E2F activity in a cell in which is its present. (I) has at least 70% homology to a sequence selected from: ARAAMAPPRGGAAAAATAALDLTGVHILEASSVPPLPERGGNAVQRKGAVDP, DKDRKKEKAAAPRITGWGLREYSKIVCEKVEAKGRTTYNEVADEIYSELKS,

MAHIGQGFDEKNIRRRVYDAFNVLIALRVIAKEKKEIRWMGLSNYRYEKIKKLEEV, RKELVNKIRNKKALLQEIEKQFDDLQNIKLRNQTLESSAENVNGIRLPFVLVKTSR, and KARVEIEISDDSKFAHFEFNGAPFTLHDDLSILEGVRGNSIGKAGRATLH, fully defined in the specification. Preferred Polynucleotide: (II) has a sequence complementary or antisense to S2. (II) is contained in plasmid pCLON33, deposit number CECT 5195 made on August 17th 1999 under the terms of the Budapest Treaty for the International Recognition of Microorganism Deposits for Patent Purposes of 28th April 1977 at the Coleccion Espanola de Cultivos Tipo. (II) has a sequence encoding an E2F protein or peptide, and encodes (I) fused to a sequence encoding for a protein label. (II) is under the control of the same regulatory element or elements. (V) is 30-100, preferably 10-20 bases long. Preferred Antibody: (XI) is capable of specifically binding with plant DP factor peptides or proteins, but not to the human, mouse or Xenopus DP. Preferred Material: (XII) comprises a peptide or protein having DNA binding activity with respect to plant DNA E2F transcription factor binding sites, where the peptides or proteins are wheat peptides or proteins and its functional variants, or parts. (XII) is in the form of a coating or otherwise bound form on a support material, or in the form of particles of the peptides or proteins and/or hetero-dimers or trimers.

ABEX UPTX: 20010515

SPECIFIC SEQUENCES - (II) has a sequence S2 comprising 1089 base pairs fully defined in the specification (claimed).

EXAMPLE - In order to isolate protein partners for wheat (Triticum monococcum) TmE2F, E2F expressed from cDNA described in PCT/EP99/03158 was fused to the Ga14-DNA binding domain and applied to yeast two-hybrid screening. The TmE2F clone by itself transactivated the reporter genes and could not be used as bait. Therefore the last 85 amino acids of TmE2F (which, based on amino acid homology studies and binding experiments, should contain the Rb-binding and trans-activation domains but not a putative dimerization domain), were deleted. This C-terminally truncated TmE2F(1-373) did not trans-activate the reporter genes in yeast and was used as a bait in a screening of a wheat cDNA library constructed as a fusion to the Gal4-activation domain. The positive clones were allowed to grow, and thirty clones were isolated. Six clones with identical DNA sequence were identified and isolated by partial DNA sequencing. These sequences were used as a query in a BLAST search against GENBANK database, and the already identified members of the animal E2F-dimerization partner (DP) family were retrieved. The TmDP cDNA clone isolated by two-hybrid screen was 1089 base pairs long, including the poly-A tract, and contained a 5'-untranslated region and a 233 base pair 3'-untranslated region. The cDNA clone contained a single open reading frame of 261 amino acids.

L116 ANSWER 5 OF 5 WPIX COPYRIGHT 2003 THOMSON DERWENT on STN
AN **1991-132863** [18] WPIX
DNC **C1991-057339**
TI **Cell free** synthesis and isolation of novel genes - which are semi-random sequence and bind to e.g. antibodies or toxins.
DC B04 D16
IN KAWASAKI, G; KAWASAKI, G H
PA (KAWA-I) KAWASAKI G; (OPTE-N) OPTEIN INC
CYC 19
PI WO 9105058 A 19910418 (199118)* 52p
RW: AT BE CH DE DK ES FR GB IT LU NL SE
W: AU CA JP KR NO
AU 9065373 A 19910428 (199131)
EP 494955 A1 19920722 (199230) EN 52p C12P021-00
R: AT BE CH DE DK ES FR GB IT LI LU NL SE
JP 05503000 W 19930527 (199326) 18p C12P021-00
AU 638762 B 19930708 (199334) C12N015-10
EP 494955 A4 19920812 (199523)
US 5643768 A 19970701 (199732) 14p C12P019-34
US 5658754 A 19970819 (199739) 12p C12P021-02

EP 494955 B1 19980715 (199832) EN C12P021-00
R: AT BE CH DE DK ES FR GB IT LI LU NL SE
DE 69032483 E 19980820 (199839) C12P021-00
ES 2118066 T3 19980916 (199848) C12P021-00
KR 204359 B1 19990615 (200063) C12N015-00
KR 204360 B1 19990615 (200063) C12N015-00
JP 3127158 B2 20010122 (200112) 17p C12P021-00
KR 185192 B1 19990401 (200113) C12N015-10
JP 2001078787 A 20010327 (200122) 17p C12N015-09
CA 2067194 C 20030318 (200325) EN C12N015-10

ADT EP 494955 A1 EP 1990-915566 19901004, WO 1990-US5682 19901004; JP 05503000 W JP 1990-514372 19901004, WO 1990-US5682 19901004; AU 638762 B AU 1990-65373 19901004; EP 494955 A4 EP 1990-915566 ; US 5643768 A Cont of US 1989-417357 19891005, Div ex US 1991-798985 19911129, US 1995-469026 19950606; US 5658754 A Cont of US 1989-417357 19891005, US 1991-798985 19911129; EP 494955 B1 EP 1990-915566 19901004, WO 1990-US5682 19901004; DE 69032483 E DE 1990-632483 19901004, EP 1990-915566 19901004, WO 1990-US5682 19901004; ES 2118066 T3 EP 1990-915566 19901004; KR 204359 B1 WO 1990-US5682 19901004, Div ex KR 1992-700769 19920404, KR 1998-708672 19981028; KR 204360 B1 WO 1990-US5682 19901004, Div ex KR 1992-700769 19920404, KR 1998-708673 19981028; JP 3127158 B2 JP 1990-514372 19901004, WO 1990-US5682 19901004; KR 185192 B1 WO 1990-US5682 19901004, KR 1992-700769 19920404; JP 2001078787 A Div ex JP 1990-514372 19901004, JP 2000-240298 19901004; CA 2067194 C CA 1990-2067194 19901004, WO 1990-US5682 19901004

FDT EP 494955 A1 Based on WO 9105058; JP 05503000 W Based on WO 9105058; AU 638762 B Previous Publ. AU 9065373, Based on WO 9105058; EP 494955 B1 Based on WO 9105058; DE 69032483 E Based on EP 494955, Based on WO 9105058; ES 2118066 T3 Based on EP 494955; JP 3127158 B2 Previous Publ. JP 05503000, Based on WO 9105058; CA 2067194 C Based on WO 9105058

PRAI US 1989-417357 19891005; US 1991-798985 19911129; US 1995-469026 19950606

REP 2.Jnl.Ref; GB 2183661; No-Citns.; UK 2183661

IC C07H015-12; C07K007-00; C12N015-10; C12P019-34; C12P021-02
ICM C12N015-00; C12N015-09; C12P019-34; C12P021-00; C12P021-02
ICS C07H015-12; C07K007-00; C12N015-11; C12R001:19; C12R001:91; G01N033-15; G01N033-50; G01N033-566

ICA C12N015-10

ICI C12P021-00, C12R001:91

AB WO 9105058 A UPAB: 19940223

Novel polypeptides (I) prodn. comprises construction of an in vitro expression unit (II) (contg. a 5' untranslated region with an RNA polymerase binding sequence, a ribosome binding sequence and a translation initiation region), attaching it to at least one semi-random nucleotide sequence and expressing or replication associated sequences followed by translation of the RNA produced to produce polysomes. The polysomes are bound to a substance of interest, isolated, disrupted to release the RNA which is recovered and converted to a cDNA which can be expressed as a novel polypeptide.

Also claimed are: 1. (I) where the RNA is translated into biologically active peptides or sub divided in such a way that the gene of interest is isolated and used to construct a cDNA to express the novel polypeptides; 2. (I) as above where the RNA is subdivided after translation into active polypeptides; 3. Isolation of a nucleotide sequence encoding a polypeptide of interest comprising transcribing (II) and at least one semi-random nucleotide to produce a mRNA library which is translated to produce polypeptides still attached to polysomes, and the polysomes are contacted to a substance of interest and the mRNA is recovered from the bound polysome.

USE - The method allows the isolation of semi-random DNA or RNA sequences that encode novel polypeptides which can bind specific substances. @(52pp Dwg.No.0/0)

0/0

FS CPI
FA AB
MC CPI: B04-B04A1; B04-C01; D05-C12; D05-H12
ABEQ JP 05503000 W UPAB: 19931116
Polypeptides (I) prodn. comprises construction of an in vitro expression unit (II) (contg. a 5' untranslated region with an RNA polymerase binding sequence, a ribosome binding sequence and a translation initiation region), attaching it to at least one semi-random nucleotide sequence and expressing or replication associated sequences followed by translation of the RNA produced to produce polysomes.

The polysomes are bound to a substance of interest, isolated, disrupted to release the RNA which is recovered and converted to a cDNA which can be expressed as a novel polypeptide.

USE - The method allows the isolation of semi-random DNA or RNA sequences that encode novel polypeptides which can bind specific substances.

ABEQ US 5643768 A UPAB: 19970806
A method for isolating a nucleotide sequence which encodes a polypeptide of interest, comprising

transcribing an expression unit which comprises a 5' untranslated region containing an RNA polymerase binding sequence, a ribosome binding sequence, a translation initiation signal, and one or more semi-random nucleotide sequences to produce a mRNA library;

translating in vitro the mRNA library under conditions which maintain polysomes having polypeptide chains attached thereto; contacting the polysomes to a substance of interest and isolating mRNA from the polysomes that specifically bind to the substance of interest.

Dwg.0/0

ABEQ US 5658754 A UPAB: 19970926
A method for producing novel polypeptides, comprising:

(a) constructing an in vitro expression unit comprising a 5 untranslated region containing an RNA polymerase binding site, a ribosome binding site, and a translation initiation signal, said expression unit being capable of producing mRNA;

(b) attaching one or more semi-random polynucleotides to said expression unit;

(c) transcribing or replicating from the RNA polymerase binding site the polynucleotides associated with the expression unit and semi-random polynucleotides to produce mRNA;

(d) translating said mRNA to produce polysomes under conditions sufficient to maintain said polysomes;

(e) binding said polysomes to a substance of interest;

(f) isolating said polysomes that bind to said substance of interest;

(g) disrupting said isolated polysomes to release mRNA;

(h) recovering said mRNA;

(i) constructing cDNA from said recovered mRNA; and

(j) expressing said cDNA to produce novel polypeptides.

Dwg.0/0

=> d his

(FILE 'HOME' ENTERED AT 09:09:12 ON 22 JUL 2003)
SET COST OFF

FILE 'HCAPLUS' ENTERED AT 09:09:23 ON 22 JUL 2003

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		E SEKI E/AU
L2	3	S E3,E6
		E KIGAWA T/AU
L3	46	S E3,E4
		E YOKOYAMA S/AU
L4	658	S E3-E5,E51

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                E KIKAWA T/AU
L5           11 S E3,E7
L6          667 S L2-L5
L7          287 S L6 AND PROTEIN
L8           45 S L7 AND CELL(L)FREE
L9            2 S L8 AND FUS?
L10          45 S L7 AND (BIOCHEM?(L)METHOD?)/SC,SX
L11          20 S L8 AND L10
                E TRANSLATION/CT
                E E3+ALL
L12       16019 S E1
                E E2+ALL
L13       32323 S E3,E2
L14       16971 S E2+NT
L15          39 S L7 AND L12-L14
L16          24 S L8 AND L15
L17          30 S L11,L16
L18          63 S L6 AND P/DT
L19          10 S L18 AND L17
L20          53 S L18 NOT L19
L21          20 S L17 NOT L19
L22          30 S L19,L21
L23          50 S L8-L11,L15 NOT L16-L22
                SEL DN AN 20 21
L24           2 S L23 AND E1-E6
L25          32 S L22,L24
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L26        6957 S E2,E1+NT
                E E16+ALL
L27      960080 S E2,E3,E1+NT
                E CRYSTAL STRUCTURE/CT
                E E8+ALL
L28        6204 S E1
                E E2+ALL
L29        3065 S E1+NT
                E E10+ALL
L30      325985 S E1,E2
L31      304433 S L26-L30 AND PROTEIN
                E FUSION/CT
                E E26+ALL
L32       13851 S E2,E3,E1+NT
L33        5794 S L31 AND L32
L34          15 S L33 AND CELL FREE
L35          56 S L33 AND CELL(L)FREE
L36       14689 S PROTEIN#/SC,SX,CW,BI AND CELL FREE
L37       45380 S PROTEIN#/SC,SX,CW,BI AND CELL(L)FREE
L38         203 S PROTEIN#/SC,SX,CW,BI AND CELLFREE?
L39        1761 S L36-L38 AND FUS?
L40         232 S L32 AND L39
L41        1761 S L39,L40
L42         146 S L41 AND (BIOCHEM?(L)METHOD?)/SC,SX
L43         461 S L41 AND 3/SC,SX
L44          58 S L42,L43 AND (GFP OR GREEN FLUORESCEN?)
L45           2 S L43 AND (3D OR (3 OR THREE OR THIRD)()(D OR DIM OR DIMENSION?
L46         100 S L12-L14 AND L41
L47           2 S L46 AND (3D OR (3 OR THREE OR THIRD)()(D OR DIM OR DIMENSION?
L48          37 S L43 AND L46
L49          34 S L25,L45,L47
L50         148 S L44,L46,L48 NOT L49
                SEL DN AN 4-7 10 13 51 57 67 73 76 106
L51          12 S L50 AND E1-E36
L52          46 S L49,L51
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L53          184 S L42,L43 AND ("E" OR ESCH?)()COLI
L54           43 S L53 AND TRANSLAT?
                 SEL DN AN 2 3 7 8 10 13 14 20
L55            8 S L54 AND E37-E60
L56           54 S L53 AND TRANSCRI?
L57           31 S L56 NOT L54
L58           50 S L52,L55
L59           50 S L58 AND L1-L58
L60           50 S L59 AND (CELL FREE OR FUS? OR SYNTH? OR VECTOR OR EXPRESS? OR

     FILE 'HCAPLUS' ENTERED AT 09:58:46 ON 22 JUL 2003

     FILE 'WPIX' ENTERED AT 10:59:22 ON 22 JUL 2003
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L61             1 S E3,E4
L62          3806 S C12N015-62/IC,ICM,ICS
L63          7778 S C12N015-11/IC,ICM,ICS
L64         22927 S C12N015-09/IC,ICM,ICS
L65           210 S L62 AND L63 AND L64
L66           167 S L65 AND C07K/IC,ICM,ICS
L67            89 S L65 AND C07K019/IC,ICM,ICS
L68           146 S L65 AND C12P021/IC,ICM,ICS
L69            69 S L67 AND L68
L70           131 S L66 AND L68
L71           131 S L69,L70
L72             1 S L71 AND (GFP OR GREEN FLUORES?)/BIX
L73             1 S L71 AND G01N024/IC,ICM,ICS
L74             2 S L71 AND G01N023/IC,ICM,ICS
L75           129 S L71 NOT L72-L74
L76             5 S L71 AND (CELL(S)FREE)/BIX
L77           271 S (CELL FREE OR CELLFREE)/BIX AND L62-L64
L78            69 S L77 AND L63
L79            19 S L78 AND C12P021/IC,ICM,ICS,ICA,ICI
                 SEL DN AN 17 18
L80             2 S E1-E4
L81            49 S L78 AND C07K/IC,ICM,ICS,ICA,ICI
L82            33 S L81 NOT L79
L83            17 S L78 NOT L79-L82
L84           125 S L75 NOT L76,L78-L83
                 SEL DN AN 22
L85             1 S L84 AND E5-E7
L86             9 S L61,L72,L74,L76,L80,L85
                 E KIGAWA T/AU
L87             8 S E3
                 E SEKI E/AU
L88            19 S E3
                 E YOKOYAMA S/AU
L89           340 S E3,E4
                 E KIKAWA/AU
L90             9 S E7
L91           371 S L87-L90
L92             8 S L91 AND C07K/IC,ICM,ICS,ICA,ICI
L93            16 S L91 AND C12N/IC,ICM,ICS,ICA,ICI
L94            18 S L91 AND G01N/IC,ICM,ICS,ICA,ICI
L95             9 S L91 AND C12P/IC,ICM,ICS,ICA,ICI
L96             8 S L91 AND C12Q/IC,ICM,ICS,ICA,ICI
L97            30 S L92-L96
                 SEL DN AN 5 6 7
L98             3 S L97 AND E1-E7
L99         12205 S (CELLFREE OR CELL(S)FREE)/BIX
L100         1925 S L99 AND C07K/IC,ICM,ICS,ICA,ICI
L101         1277 S L99 AND C12P021/IC,ICM,ICS,ICA,ICI
L102         2400 S L100,L101
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L103        1308 S L102 AND C12N015/IC,ICM,ICS,ICA,ICI
L104         113 S L103 AND C12N015-11/IC,ICM,ICS,ICA,ICI
L105         549 S L103 AND C12N015-09/IC,ICM,ICS,ICA,ICI
L106          47 S L104 AND L105
                 SEL DN AN 1 9
L107           2 S L106 AND E8-E11
L108          65 SS C12N015-70/IC,ICM,ICS,ICA,ICI AND L102
L109          65 S L108 AND L103-L105
L110           4 S L98,L107
L111           1 S L86 AND L110
L112           4 S L110,L111
L113           8 S L86 NOT L112
L114           6 S L113 AND L99
                 SEL DN AN 4
L115           1 S L114 AND E12-E13
L116           5 S L112,L115
L117           2 S L86 NOT L114-L116

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FILE 'WPIX' ENTERED AT 11:47:29 ON 22 JUL 2003

FILE 'WPIX' ENTERED AT 11:48:13 ON 22 JUL 2003
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L1             1 S US20020142387/PN
               E SEKI E/AU
L2             3 S E3,E6
               E KIGAWA T/AU
L3            46 S E3,E4
               E YOKOYAMA S/AU
L4           658 S E3-E5,E51
               E KIKAWA T/AU
L5            11 S E3,E7
L6           667 S L2-L5
L7           287 S L6 AND PROTEIN
L8            45 S L7 AND CELL(L)FREE
L9             2 S L8 AND FUS?
L10           45 S L7 AND (BIOCHEM?(L)METHOD?)/SC,SX
L11           20 S L8 AND L10
               E TRANSLATION/CT
               E E3+ALL
L12        16019 S E1
               E E2+ALL
L13        32323 S E3,E2
L14        16971 S E2+NT
L15           39 S L7 AND L12-L14
L16           24 S L8 AND L15
L17           30 S L11,L16
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L20           53 S L18 NOT L19
L21           20 S L17 NOT L19
L22           30 S L19,L21
L23           50 S L8-L11,L15 NOT L16-L22
               SEL DN AN 20 21
L24            2 S L23 AND E1-E6
L25           32 S L22,L24
               E MOLECULAR STRUCTURE/CT
               E E20+ALL
L26         6957 S E2,E1+NT
               E E16+ALL
L27       960080 S E2,E3,E1+NT
               E CRYSTAL STRUCTURE/CT
               E E8+ALL
L28         6204 S E1
               E E2+ALL
L29         3065 S E1+NT
               E E10+ALL
L30       325985 S E1,E2
L31       304433 S L26-L30 AND PROTEIN
               E FUSION/CT
               E E26+ALL
L32        13851 S E2,E3,E1+NT
L33         5794 S L31 AND L32
L34           15 S L33 AND CELL FREE
L35           56 S L33 AND CELL(L)FREE
L36        14689 S PROTEIN#/SC,SX,CW,BI AND CELL FREE
L37        45380 S PROTEIN#/SC,SX,CW,BI AND CELL(L)FREE
L38          203 S PROTEIN#/SC,SX,CW,BI AND CELLFREE?
L39         1761 S L36-L38 AND FUS?
L40          232 S L32 AND L39
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L41         1761 S L39,L40
L42          146 S L41 AND (BIOCHEM?(L)METHOD?)/SC,SX
L43          461 S L41 AND 3/SC,SX
L44           58 S L42,L43 AND (GFP OR GREEN FLUORESCEN?)
L45            2 S L43 AND (3D OR (3 OR THREE OR THIRD)()(D OR DIM OR DIMENSION?
L46          100 S L12-L14 AND L41
L47            2 S L46 AND (3D OR (3 OR THREE OR THIRD)()(D OR DIM OR DIMENSION?
L48           37 S L43 AND L46
L49           34 S L25,L45,L47
L50          148 S L44,L46,L48 NOT L49
                 SEL DN AN 4-7 10 13 51 57 67 73 76 106
L51           12 S L50 AND E1-E36
L52           46 S L49,L51
L53          184 S L42,L43 AND ("E" OR ESCH?)()COLI
L54           43 S L53 AND TRANSLAT?
                 SEL DN AN 2 3 7 8 10 13 14 20
L55            8 S L54 AND E37-E60
L56           54 S L53 AND TRANSCRI?
L57           31 S L56 NOT L54
L58           50 S L52,L55
L59           50 S L58 AND L1-L58
L60           50 S L59 AND (CELL FREE OR FUS? OR SYNTH? OR VECTOR OR EXPRESS? OR

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FILE COVERS 1907 - 22 Jul 2003  VOL 139 ISS 4
FILE LAST UPDATED: 21 Jul 2003  (20030721/ED)
 This file contains CAS Registry Numbers for easy and accurate
 substance identification.


=> d l60 all tot

L60  ANSWER 1 OF 50  HCAPLUS  COPYRIGHT 2003 ACS on STN
AN   2003:242431  HCAPLUS
DN   138:253827
TI   Method for increasing the solubility, expression rate
     and the activity of proteins during cell-free
     recombinant production
IN   Watzele, Manfred; Schweizer, Regina; Nemetz, Cordula; Steigerwald, Robin;
     Emrich, Thomas; Zaiss, Katrin; Fernholz, Erhard; Walckhoff, Baerbel;
     Schoenfeld, Hans Joachim; Offen, Birgit
PA   Roche Diagnostics GmbH, Germany; F. Hoffmann-La-Roche AG
SO   PCT Int. Appl., 32 pp.
     CODEN: PIXXD2
DT   Patent
LA   German
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IC ICM C12N
CC 16-1 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): **3**
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2003025116	A2	20030327	WO 2002-EP10329	20020914
W: JP, US				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR				
DE 10145694	A1	20030403	DE 2001-10145694	20010917

PRAI DE 2001-10145694 A 20010917

AB The invention relates to a method for producing a lysate contg. auxiliary **proteins**. According to said method, a strain, which is suitable for obtaining in vitro **translation** lysates is transformed using a **vector** contg. one or more genes that code for one or more auxiliary **proteins**, the auxiliary **proteins** being **expressed** in said strain and the lysate contg. auxiliary **proteins** being obtained from said strains. The invention also relates to a lysate contg. auxiliary **proteins** that can be obtained according to the inventive method, to blends of said lysates and to the use of the lysates and blends in in vitro **translation** systems. Thus, the inclusion of genes for the chaperonins DnaK, GrpE, and DnaK in an **Escherichia coli** strain that **expresses** human telomerase resulted in increased prodn. of **sol. protein** in a **cell-free** system.

ST Escherichia **cell free translation** chaperonin **recombinant protein**

IT Bioreactors
(CECF or CFCF; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Chaperonins
RL: BCP (Biochemical process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(DnaJ; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Chaperonins
RL: BCP (Biochemical process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(DnaK; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, animal
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(EPO; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Reporter gene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**GFP**; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Chaperonins
RL: BCP (Biochemical process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(GroEL; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Chaperonins
RL: BCP (Biochemical process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(GroES; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT **Proteins**
RL: BCP (Biochemical process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(GrpE; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MAL; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT **Proteins**
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(MBP (maltose-binding **protein**), **fusion** with erythropoietin; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, animal
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TEL; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT **Transcription**, genetic
Translation, genetic
(**cell-free**; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(dnaJ; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(dnaK; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT **Proteins**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(**green fluorescent**; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(groEL; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(groES; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(grpE; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT **Escherichia coli**
(lysates of; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Genetic **vectors**
Solubility
(method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Chaperonins
RL: BCP (Biochemical process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT **Fusion proteins (chimeric proteins)**
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Gene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Plasmid **vectors**
(pIVEX2.3-**GFP**; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Plasmid **vectors**
(pIVEX2.4-Rhodanase; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Plasmid **vectors**
(pIVEX2.4b-Mal-Epo; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Plasmid **vectors**
(pIVEX2.4bNde-hTERT; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Plasmid **vectors**
(pRDKJG; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Plasmid **vectors**
(pREP4-groESL; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Human
(**proteins**, **expression** of; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT **Proteins**
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(**recombinant**; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)
IT Gene, animal
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(rho; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT **Proteins**
RL: BCP (Biochemical process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(trigger factors; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT 95076-93-0
RL: BCP (Biochemical process); CAT (Catalyst use); BIOL (Biological study); PROC (Process); USES (Uses)
(bovine mitochondrial; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT 9001-92-7, Protease 9001-99-4, RNase 37228-74-3, Exonuclease
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**cells** that have low levels or are deficient in; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT 11096-26-7DP, Erythropoietin, **fusion protein** with maltose-binding **protein** 120178-12-3P, Telomerase
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(human; method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT 9026-04-4P, Rhodanase
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

IT 37318-49-3, **Protein** disulfide isomerase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(method for increasing **soly.**, **expression** rate and activity of **proteins** during **cell-free recombinant** prodn.)

L60 ANSWER 2 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2003:187867** HCAPLUS
DN **138:201358**
TI Nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation
IN Nemoto, Naoto
PA Mitsubishi Chemical Corp., Japan
SO Jpn. Kokai Tokkyo Koho, 16 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
IC ICM C12N015-09
ICS C12P021-02
CC **9-15** (**Biochemical Methods**)
Section cross-reference(s): **3**, 16
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2003070483	A2	20030311	JP 2001-270323	20010906

PRAI JP 2001-270323 20010906

AB A method for ligating nucleic acid via primer-less PCR using a pair of complementary and overlapping single-stranded or double-stranded DNA, is

disclosed. One of the DNA codes for a desired **protein** and the other codes for an .alpha.-helix contg. globular **protein** as support for **expressing** or displaying **proteins** or peptides as **fusion protein** in **cell-free** translation system. DNA polymerase, Taq polymerase, in particular, is used for PCR and RNA polymerase is used for transcription. DNase is used to degrade contaminating DNA. Nucleic acid derivs. such as puromycin, 3-N-aminoacylpuromycin aminonucleoside (PANS-amino acid), or 3'-N-aminoacyl adenosine aminonucleosides (AANS-amino acid), are linked to **fusion protein** coding mRNA via a linker (spacer). Polyethylene or polyethylene glycol may be used as spacer. A mutated POU-specific **domain** of transcription factor Oct-1 having a Cys residue replaced by Ala, was used as support for **expression** of peptide library in **cell-free** translation system. Carbohydrate-binding peptides, binding N-acetylglucosamine in the presence of calcium was screened from the peptide library.

ST nucleic acid ligation primer less PCR; **fusion protein expression cell free** translation

IT Nucleoside analogs
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(3-N-aminoacylpuromycin amino, as nucleic acid deriv.; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT Nucleoside analogs
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(3'-N-amino acyl adenosine amino, as nucleic acid deriv.; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT Transcription factors
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(NF-III (nuclear factor III), POU-specific **domain** of; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT Nucleic acids
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(analogs; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT **Translation, genetic**
(**cell-free**; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(double-stranded; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT **Proteins**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(globular; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(linker; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free**

translation)
IT PCR (polymerase chain reaction)
Peptide library
Protein sequences
Transcription, genetic
(nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT **Fusion proteins (chimeric proteins)**
Peptides, preparation
Proteins
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT Nucleic acids
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT Polyoxyalkylenes, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(single-stranded; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT **Proteins**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(.alpha.-helix-contg.; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT 53-79-2, Puromycin 53-79-2D, Puromycin, 3-N-aminoacyl aminonucleoside 58-61-7D, Adenosine, 3'-N-aminoacyl aminonucleosides
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(as nucleic acid deriv.; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT 9003-98-9, DNase
RL: BUU (Biological use, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses)
(for degrading contaminating DNA; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT 25322-68-3, Polyethylene glycol
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)
IT 500256-17-7 500256-18-8 500256-19-9 500256-20-2 500256-21-3 500256-22-4 500256-23-5 500256-24-6
RL: PRP (Properties)
(unclaimed nucleotide sequence; nucleic acid ligation via primer-less

PCR for **fusion protein expression** in **cell-free** translation)

IT 500256-25-7
RL: PRP (Properties)
(unclaimed **protein** sequence; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT 9002-88-4, Polyethylene
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use as linker; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT 9012-90-2, DNA polymerase
RL: BUU (Biological use, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses)
(use for PCR; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

IT 9014-24-8, RNA polymerase
RL: BUU (Biological use, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses)
(use for transcription; nucleic acid ligation via primer-less PCR for **fusion protein expression** in **cell-free** translation)

L60 ANSWER 3 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2003:187866** HCAPLUS
DN **138:201357**
TI Globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system
IN Nemoto, Naoto
PA Mitsubishi Chemical Corp., Japan
SO Jpn. Kokai Tokkyo Koho, 15 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
IC ICM C12N015-09
ICS C07K014-00; C07K019-00; C12P021-02
CC **9-15** (**Biochemical Methods**)
Section cross-reference(s): **3**, 16
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2003070482	A2	20030311	JP 2001-270322	20010906
PRAI JP 2001-270322		20010906		

AB Use of .alpha.-helix contg. globular **protein** as support for **expressing** or displaying **proteins** or peptides as **fusion protein** in **cell-free** translation system, is disclosed. Nucleic acid derivs. such as puromycin, 3-N-aminoacylpuromycin aminonucleoside (PANS-amino acid), or 3'-N-aminoacyl adenosine aminonucleosides (AANS-amino acid), are linked to **fusion protein** coding mRNA via a linker (spacer). Polyethylene or polyethylene glycol may be used as spacer. A mutated POU-specific **domain** of transcription factor Oct-1 having a Cys residue replaced by Ala, was used as support for **expression** of peptide library in **cell-free** translation system. Carbohydrate-binding peptides, binding N-acetylglucosamine in the presence of calcium was screened from the peptide library.

ST globular **protein** support displaying **fusion protein cell free** translation

IT Nucleoside analogs

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(3-N-aminoacylpuromycin amino, as nucleic acid deriv.; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT Nucleoside analogs
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(3'-N-amino acyl adenosine amino, as nucleic acid deriv.; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT Transcription factors
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(NF-III (nuclear factor III), POU-specific **domain** of; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT Nucleic acids
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(analogs; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT **Translation, genetic**
(**cell-free**; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT Peptide library
Protein sequences
(globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT **Fusion proteins (chimeric proteins)**
Peptides, preparation
Proteins
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT Polyoxyalkylenes, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT **Proteins**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(globular; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT **Proteins**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(.alpha.-helix-contg.; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT 500252-38-0
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological

study); USES (Uses)
(amino acid sequence; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT 53-79-2, Puromycin 53-79-2D, Puromycin, 3-N-aminoacyl aminonucleoside 58-61-7D, Adenosine, 3'-N-aminoacyl aminonucleosides
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(as nucleic acid deriv.; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT 25322-68-3, Polyethylene glycol
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT 500256-26-8 500256-27-9 500256-28-0 500256-29-1 500256-30-4 500256-31-5 500256-32-6 500256-33-7
RL: PRP (Properties)
(unclaimed nucleotide sequence; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

IT 9002-88-4, Polyethylene
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use as linker; globular **protein** as support for **expressing** or displaying **fusion proteins** in **cell-free** translation system)

L60 ANSWER 4 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2003:150699** HCAPLUS
DN **138:199905**
TI Method for determining gene **expression** in a **cell-free transcription/translation** system
IN Erdmann, Volker A.; Lamla, Thorsten; Stiege, Wolfgang
PA Germany
SO Ger. Offen., 8 pp.
CODEN: GWXXBX
DT Patent
LA German
IC ICM C12N015-00
ICS C12M001-42; C12P021-00
CC **3-1** (Biochemical Genetics)
Section cross-reference(s): **9**
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 10137792	A1	20030227	DE 2001-10137792	20010806
WO 2003016526	A1	20030227	WO 2002-DE2672	20020717

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI DE 2001-10137792 A 20010806
AB The invention relates to a method using a flow reaction chamber for studying gene **expression** in a **cell-free**

transcription/translation system. The reaction soln. contains all necessary components of the **transcription/translation** app., amino acids, nucleotides, energy supplying and metab. components necessary for the **synthesis**. During **translation** the produced **proteins** are immobilized on a matrix.

ST gene **expression** detection **cell free transcription translation** system

IT **Escherichia coli**
(S30 D10, lysate of; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Pumps
(centrifugal, for maintaining the reaction flow in the reactor; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Amino acids, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(component of reaction mix; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Gene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**expression**; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT UV and visible spectroscopy
(for detg. **protein** concn.; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Matrix media
(for specific **protein** binding, hydrophilic/hydrophobic, antigen/antibody, metal ion; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Antibodies
Antigens
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for specific **protein** binding; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT **Transcription**, genetic
Translation, genetic
(method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT **Fusion proteins (chimeric proteins)**
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Immobilization, molecular
(of **protein** products; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Reactors
(reaction chamber of **transcription/translation** system; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT Membranes, nonbiological
(semipermeable, component of the reactor; method for detg. gene **expression** in a **cell-free transcription/translation** system)

IT 147395-23-1 205938-74-5
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; method for detg. gene **expression** in a **cell-free transcription/translation** system)
IT 50812-37-8, Glutathione-S-transferase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(as **protein** N- or C-terminal **fusion** partner; method for detg. gene **expression** in a **cell-free transcription/translation** system)
IT 56-65-5, ATP, biological studies 63-39-8, UTP 65-47-4, CTP 86-01-1, GTP 2466-09-3, Diphosphoric acid
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(component of reaction mix; method for detg. gene **expression** in a **cell-free transcription/translation** system)
IT 9013-20-1, Streptavidin
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for binding WSHPQFEK and AWRHPQFGG amino acid sequences; method for detg. gene **expression** in a **cell-free transcription/translation** system)
IT 71-00-1, Histidine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(frequency of in **protein** N-terminal or C-terminal; method for detg. gene **expression** in a **cell-free transcription/translation** system)
IT 15158-11-9, Cu 2+, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(matrix component; method for detg. gene **expression** in a **cell-free transcription/translation** system)
IT 1306-06-5, Hydroxyapatite
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(matrix; method for detg. gene **expression** in a **cell-free transcription/translation** system)
IT 14701-22-5, Nickel 2+, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(method for detg. gene **expression** in a **cell-free transcription/translation** system)
RE.CNT 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Anon; Nucl Acids Red 2001, V29(15), Pe73

L60 ANSWER 5 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2003:133583** HCAPLUS
DN **138:183528**
TI Method for detecting interaction between substance and **protein** using **cell-free** translation system, method for screening **protein** interacting with substance, and method for forming complex of substance and **protein** interacting with the substance
IN Yanagawa, Hiroshi; Miyamoto, Etsuko; Matsumura, Nobutaka; Doi, Nobuhide; Tateyama, Seiji; Ishizaka, Masamichi; Horisawa, Kenichi
PA Keio University, Japan
SO PCT Int. Appl., 114 pp.
CODEN: PIXXD2
DT Patent
LA Japanese

IC ICM G01N033-50
ICS G01N033-15; G01N033-566
CC **9-16** (**Biochemical Methods**)
Section cross-reference(s): **3**, 6
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2003014734	A1	20030220	WO 2002-JP8078	20020807

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI	JP 2001-239175	A	20010807
	JP 2002-172129	A	20020612

AB A method for detecting an interaction between a substance (bait) and a **protein** (prey) using a **cell-free** translation system is provided, in which the bait is brought contact with the prey, and the complex formed by the contact is detected. In this method, a **fusion protein** of the pray with a **protein** usable as a label for detection is designed while the bait is added with a modification for sepn. Then, mRNA encoding the **fusion protein** contg. the pray is translated in a **cell-free** translation system in the presence of the bait, and thereby, the translation step is coupled with the interaction step. Thus, the prey is formed in the **cell-free** translation system, and then, brought into contact with the bait. The complex between the bait and the prey is detected with the use of the modification for sepn. and the label for detection. Thus, the anal. of an interaction between **proteins**, between a nucleic acid and a **protein**, or else is performed in vitro at a high sensitivity. In case where the bait is a **protein**, alternatively, mRNA encoding the **fusion protein** contg. the bait and mRNA encoding the **protein** contg. the prey or its **fusion protein** are translated in a **cell-free** cotranslation system, and thereby, the translation are coupled with the interaction step. Thus, the bait and prey formed are allowed to interact with each other, and the complex is detected with the use of the modification for sepn. and the label for detection. Thereby, the anal. of an interaction between **proteins**, between a nucleic acid and a **protein**, or else is performed in vitro at a high sensitivity.

ST **protein** interaction **cell free** translation system; in vivo virus STABLE **protein** DNA interaction

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(DNA-binding; methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT **Translation, genetic**
(**cell-free**; methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT Nucleic acid library
(dT priming cDNA; methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT Molecular association

(methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT Avidins
Fusion proteins (chimeric proteins)
Nucleic acids
Proteins
Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT mRNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT Nucleic acid library
(random priming cDNA; methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT **Recombination**, genetic
(translocation; methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

IT 53-79-2, Puromycin 58-85-5, Biotin 9013-20-1, Streptavidin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for detecting **protein-protein** or **protein**-nucleic acid interaction using **cell-free** translation system)

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD

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(1) Keio University; WO 0248347 A 2002 HCAPLUS
(2) Keio University; JP 2002176987 A 2002 HCAPLUS
(3) Mitsubishi Chemical Corp; US 6361943 A 1998 HCAPLUS
(4) Mitsubishi Chemical Corp; EP 962527 A 1998 HCAPLUS
(5) Mitsubishi Chemical Corp; WO 9816636 A 1998 HCAPLUS
(6) Mitsubishi Chemical Corp; JP 11-322781 A 1999 HCAPLUS
(7) Mitsubishi Chemical Corp; JP 2000139468 A 2000 HCAPLUS
(8) Mitsubishi Chemical Corp; JP 2001128690 A 2001 HCAPLUS
(9) The Institute Of Physical And Chemical Research; WO 0068690 A 2001 HCAPLUS
(10) The Institute Of Physical And Chemical Research; EP 1182458 A 2001 HCAPLUS
(11) The Institute Of Physical And Chemical Research; JP 2001027633 A 2001 HCAPLUS

L60 ANSWER 6 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:133461 HCAPLUS
DN 138:182052
TI Engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system
IN Kiga, Daisuke; Sakamoto, Kensaku; Hirao, Ichiro; **Yokoyama, Shigeyuki**
PA Japan Science and Technology Corporation, Japan
SO PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DT **Patent**
LA Japanese
IC ICM C12N015-52
ICS C12N009-00; C07B061-00; G01N033-48; G06F017-30; G06F017-50
CC 3-2 (Biochemical Genetics)

Section cross-reference(s): 7, **9**, 16

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003014354	A1	20030220	WO 2002-JP118	20020111
	W: CA, JP, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
PRAI	JP 2001-234135	A	20010801		

AB Genetically modified tyrosyl-tRNA **synthases** more efficiently incorporating unnatural amino acids, particularly 3-substituted tyrosine analogs, are provided. A method of designing such tyrosyl-tRNA **synthase** based on the three-dimensional structure anal. of the complex of tyrosyl-tRNA **synthase** and tyrosyl-AMP is also provided. Use of such modified tyrosyl-tRNA **synthases** for site-specific incorporation of an unnatural amino acid into **proteins** is also provided. Tyrosyl-tRNA **synthetase** (TyrRS) from **Escherichia coli** was engineered to preferentially recognize 3-iodo-L-tyrosine rather than L-tyrosine for the site-specific incorporation of 3-iodo-L-tyrosine into **proteins** in eukaryotic translation systems. The wild-type TyrRS does not recognize 3-iodo-L-tyrosine, because of the bulky iodine substitution. On the basis of the reported crystal structure of Bacillus stearothermophilus TyrRS, three residues, Y37, Q179, and Q195, in the L-tyrosine-binding site were chosen for mutagenesis. Thirty-four single amino acid replacements and 16 of their combinations were screened by in vitro biochem. assays. A combination of the Y37V and Q195C mutations changed the amino acid specificity in such a way that the variant TyrRS activates 3-iodo-L-tyrosine 10-**fold** more efficiently than L-tyrosine. This engineered **enzyme**, TyrRS(V37C195), was tested for use in the wheat germ **cell-free** translation system, which has recently been significantly improved, and is now as productive as conventional **recombinant** systems. During the translation in the wheat germ system, an **E. coli** suppressor tRNATyr was not aminoacylated by the wheat germ **enzymes**, but was aminoacylated by the **E. coli** TyrRS(V37C195) variant with 3-iodo-L-tyrosine. After the use of the 3-iodotyrosyl-tRNA in translation, the resultant uncharged tRNA could be aminoacylated again in the system. A mass spectrometric anal. of the produced **protein** revealed that more than 95% of the amino acids incorporated for an amber codon were iodotyrosine, whose concn. was only twice that of L-tyrosine in the translation. Therefore, the variant **enzyme**, 3-iodo-L-tyrosine, and the suppressor tRNA can serve as an addnl. set orthogonal to the 20 endogenous sets in eukaryotic in vitro translation systems.

ST engineering Escherichia tyrosyl tRNA **synthetase** unnatural amino acid translation; tyrosyl tRNA **synthetase** substrate specificity change genetic engineering; iodotyrosine incorporation tyrosyl tRNA **synthetase** Escherichia mutagenesis

IT Codons
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(amber, use in unnatural amino acid incorporation; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT **Translation, genetic**
(**cell-free**; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT Aminoacylation
Genetic engineering

Protein engineering
(engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(incorporating unnatural amino acids; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT Ras **proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(p21c-Ha-ras, incorporating unnatural amino acids; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT **Conformation**
(**protein**, of TyrRS-tyrosyl-AMP complex, **protein** engineering based on; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT Mutagenesis
(site-directed, substitution; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT **Enzyme functional** sites
(substrate-binding; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT tRNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(suppressor, use in unnatural amino acid incorporation; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT Eukaryota
Prokaryote
(transformed with modified tyrosyl-tRNA **synthase** gene; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT **Escherichia coli**
(tyrosyl-tRNA **synthetase** from; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 234771-42-7D, Tyrosyl-AMP, complex with tyrosyl-tRNA **synthetase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(3D structure of; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 52-90-4, L-Cysteine, biological studies 56-45-1, L-Serine, biological studies 70-47-3, L-Asparagine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(at position 195 in mutant; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 56-85-9, L-Glutamine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(at position 195 in wild type; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 56-41-7, L-Alanine, biological studies 61-90-5, L-Leucine, biological studies 72-18-4, L-Valine, biological studies 73-32-5, L-Isoleucine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(at position 37 in mutant; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 60-18-4, L-Tyrosine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(at position 37 in wild type; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 9023-45-4, Tyrosyl-tRNA **synthetase**
RL: BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); USES (Uses)
(engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 60-18-4D, L-Tyrosine, 3-halo-substituted analogs 60-18-4D, L-Tyrosine, 3-substituted analogs 70-78-0, 3-Iodo-L-tyrosine
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(incorporation of; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 497985-48-5 497985-49-6 497985-50-9 497985-51-0 497985-52-1
497985-53-2 497985-54-3 497985-55-4 497985-56-5 497985-57-6
497985-58-7
RL: PRP (Properties)
(unclaimed nucleotide sequence; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

IT 497985-47-4 497985-59-8
RL: PRP (Properties)
(unclaimed **protein** sequence; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in **cell-free** translation system)

RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L60 ANSWER 7 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:132002 HCAPLUS

TI Present status of **cell-free protein synthesis**
AU **Kigawa, Takanori; Yokoyama, Shigeyuki**
CS Institute of Physical and Chemical Research, Japan
SO Posutoshikuensu Tanpakushitsu Jikkenho (2002), Volume 2, 14-24. Editor(s): Oshima, Tairo. Publisher: Tokyo Kagaku Dojin, Tokyo, Japan. CODEN: 69DQE2
DT Conference
LA Japanese
CC **9** (**Biochemical Methods**)
AB Unavailable

L60 ANSWER 8 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:53294 HCAPLUS
DN 138:299270
TI **Protein expression** systems for structural genomics and proteomics
AU **Yokoyama, Shigeyuki**
CS RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama, 230-0045, Japan
SO Current Opinion in Chemical Biology (2003), 7(1), 39-43 CODEN: COCBF4; ISSN: 1367-5931
PB Elsevier Science Ltd.
DT Journal; General Review
LA English
CC 6-0 (General Biochemistry)
Section cross-reference(s): 3
AB A review. One of the key steps of structural genomics and proteomics is high-throughput **expression** of many target **proteins**. Gene **cloning**, esp. by ligation-independent **cloning** techniques, and **recombinant protein expression** using microbial hosts such as **Escherichia coli** and the yeast Pichia pastoris are well optimized and further robotized. **Cell-free protein synthesis** systems have been developed for large-scale prodn. of **protein** samples for NMR (stable-isotope labeling) and X-ray crystallog. (selenomethionine substitution). **Protein folding** is still a major bottleneck in **protein expression**. **Cell**-based and **cell-free** methods for screening of suitable samples for structure detn. have been developed for achieving a high success rate.
ST review **protein** sample **expression** system structural genomics proteomics
IT **Translation, genetic**
(**cell-free**, for **protein** sample prepn.; **protein expression** systems for structural genomics and proteomics)
IT High throughput screening
(**protein expression** systems for structural genomics and proteomics)
IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(**protein expression** systems for structural genomics and proteomics)
IT Sample preparation
(**protein** sample prepn.; **protein expression** systems for structural genomics and proteomics)
IT **Escherichia coli**
Komagataella pastoris
(**protein** sample prodn. in; **protein expression** systems for structural genomics and proteomics)
IT Genome

(structural genomics, **protein** sample prepn.; **protein expression** systems for structural genomics and proteomics)
IT Proteome
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(structural proteomics, **protein** sample prepn.; **protein expression** systems for structural genomics and proteomics)
RE.CNT 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L60 ANSWER 9 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2003:39992** HCAPLUS
DN **138:286030**
TI **Protein synthesis** and co-translational **folding** in **cell-free** translation systems
AU Spirin, Alexander S.
CS Inst. of Protein Res., Russian Acad. of Sci, Pushchino, 142292, Russia
SO Recombinant Protein Production with Prokaryotic and Eukaryotic Cells: A Comparative View on Host Physiology, Selected Articles from the Meeting of the EFB Section on Microbial Physiology, Semmering, Austria, Oct. 5-8, 2000 (2001), Meeting Date 2000, 1-15. Editor(s): Merten, Otto-Wilhelm. Publisher: Kluwer Academic Publishers, Dordrecht, Neth.
CODEN: 69DLJQ; ISBN: 0-7923-7137-2
DT Conference; General Review
LA English
CC 16-0 (Fermentation and Bioindustrial Chemistry)
AB A review, with refs. The **cell-free** methodol. for the

synthesis of **functionally** active **proteins** is considered, and the so-called continuous **cell-free** translation and transcription-translation systems are described. The continuous **cell-free** systems for gene **expression** are based on the use of a porous barrier that retains the high-mol.-wt. components of the **protein-synthesizing** machinery within a defined reaction compartment, and at the same time provides the continuous feeding with substrates (NTPs and amino acids) and the removal of reaction products. There are two versions of the continuous systems: the flow version (continuous-flow **cell-free**, or CFCF systems) and the dialysis version (continuous-exchange **cell-free**, or CECF systems). Both versions have been shown to provide a prolonged **synthesis** of **proteins**, as compared with std. (batch) **cell-free** systems, and correspondingly a significantly higher yield of **proteins synthesized**. The **synthesis** of **fusion proteins** and the direct **expression** of PCR products in **cell-free** systems are discussed as promising methodol. approaches in a no. of cases. Using the monitoring of polypeptide elongation in **cell-free** systems the evidence is presented that the **folding** of **synthesized** polypeptides into **functional protein** globules proceeds on ribosomes during translation (co-translational **protein folding**).

ST review **protein synthesis folding** **cell free** translation system

IT **Translation, genetic**
(**cell-free; protein synthesis** and co-translational **folding** in **cell-free** translation systems)

IT Biotechnology
Protein folding
(**protein synthesis** and co-translational **folding** in **cell-free** translation systems)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**protein synthesis** and co-translational **folding** in **cell-free** translation systems)

RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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L60 ANSWER 10 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:29500 HCAPLUS
DN 138:84441
TI PCR amplification of template DNA for **cell-free protein synthesis** system
IN Motoda, Yoko; Yabuki, Takashi; **Kikawa, Takanori; Yokoyama, Shigeyuki**
PA Institute of Physical and Chemical Research, Japan
SO Jpn. Kokai Tokkyo Koho, 17 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
IC ICM C12N015-09
ICS C12P021-02
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): **6, 9**
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 2003009880	A2	20030114	JP 2001-201356	20010702
	WO 2003004703	A1	20030116	WO 2002-JP6261	20020624

W: CA, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

PRAI JP 2001-201356 A 20010702
AB A process for producing template DNA for use in **cell-free protein synthesis** by DNA amplification, is provided. A first double-stranded DNA (dsDNA) coding for a **protein**, a second dsDNA overlapping at the 5' end, a third dsDNA overlapping at the 3' end, a sense primer hybridizable to the 5' end of the second dsDNA, an antisense primer hybridizable to the 3' end of the 3rd dsDNA, are used for polymerase chain reaction (PCR). Single-stranded DNA can also be used in place of dsDNA. Use of a terminator sequence and peptide tag such as maltose-binding **protein**, cellulose-binding **domain**, glutathione-S-transferase, thioredoxin, streptavidin-binding peptide, or histidine-contg. peptide, is claimed. **Expression** of Ras **protein** in **cell-free protein synthesis** system using the native histidine tag present in the N-terminal of chicken muscle lactate dehydrogenase, T7 promoter, and T7 terminator, is described. Chloramphenicol acetyltransferase, and 10 arbitrarily chosen **clones** from mouse full-length cDNA library were also **expressed.** By using this process, a desired polypeptide can be **synthesized** within a short period of time at a high yield and at a low cost, compared with the

conventional processes.
ST PCR amplification template DNA cell translation system
IT Nucleic acid amplification (method)
(DNA; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT PCR (polymerase chain reaction)
Protein sequences
(PCR amplification of template DNA for **cell-free protein synthesis** system)
IT **Proteins**
Ras **proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(PCR amplification of template DNA for **cell-free protein synthesis** system)
IT Primers (nucleic acid)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(PCR amplification of template DNA for **cell-free protein synthesis** system)
IT **Translation, genetic**
(**cell-free**; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(double-stranded; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT Peptides, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(histidine-contg., use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(single-stranded; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT Peptides, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(streptavidin-binding, use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT Peptides, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(tag; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(terminator; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT Thioredoxins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT 9040-07-7P, Chloramphenicol acetyltransferase
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP

(Preparation)
(PCR amplification of template DNA for **cell-free protein synthesis** system)
IT 140295-47-2, GenBank M21532 140531-55-1, GenBank M16206 142318-93-2, GenBank X65627 150947-97-0, GenBank L16904 175301-96-9, GenBank U51204 180770-00-7, GenBank D87663 186016-45-5, GenBank U85511 252790-02-6, GenBank S68022 384441-00-3, GenBank X13605 391530-17-9, GenBank M32599
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(Biological study)
(PCR amplification of template DNA for **cell-free protein synthesis** system)
IT 484053-89-6
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(amino acid sequence; PCR amplification of template DNA for **cell-free protein synthesis** system)
IT 484100-59-6 484100-60-9 484100-61-0 484100-62-1 484100-63-2 484100-64-3 484100-65-4 484100-66-5 484100-67-6 484100-68-7 484100-69-8 484100-70-1 484100-71-2 484100-72-3 484100-73-4 484100-74-5 484100-75-6 484100-76-7 484100-77-8 484100-78-9 484100-79-0 484100-80-3 484100-81-4
RL: PRP (Properties)
(unclaimed nucleotide sequence; pCR amplification of template DNA for **cell-free protein synthesis** system)
IT 50812-37-8, Glutathione S-transferase
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)

L60 ANSWER 11 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:29497 HCAPLUS
DN 138:84439
TI PCR amplification of template DNA for **cell-free protein synthesis** system
IN Motoda, Yoko; Yabuki, Takashi; **Kikawa, Takanori**; **Yokoyama, Shigeyuki**
PA Institute of Physical and Chemical Research, Japan
SO Jpn. Kokai Tokkyo Koho, 17 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
IC ICM C12N015-09
ICS C12P021-02
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): **6, 9**
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 2003009877	A2	20030114	JP 2001-200676	20010702
PRAI	JP 2001-200676		20010702		

AB A process for producing template DNA for use in **cell-free protein synthesis** by DNA amplification, is provided. A first double-stranded DNA (dsDNA) coding for a **protein**, a second dsDNA overlapping at the 5' end, a third dsDNA overlapping at the 3' end, a sense primer hybridizable to the 5' end of the second dsDNA, an antisense primer hybridizable to the 3' end of the 3rd dsDNA, are used for polymerase chain reaction (PCR). Single-stranded DNA can also be used in place of dsDNA. Use of a terminator sequence and peptide tag such as maltose-binding **protein**, cellulose-binding **domain**, glutathione-S-transferase, thioredoxin,

streptavidin-binding peptide, or histidine-contg. peptide, is claimed. **Expression** of Ras **protein** in **cell-free protein synthesis** system using the native histidine tag present in the N-terminal of chicken muscle lactate dehydrogenase, T7 promoter, and T7 terminator, is described. Chloramphenicol acetyltransferase, and 10 arbitrarily chosen **clones** from mouse full-length cDNA library were also **expressed.** By using this process, a desired polypeptide can be **synthesized** within a short period of time at a high yield and at a low cost, compared with the conventional processes.

ST PCR amplification template DNA cell translation system

IT Nucleic acid amplification (method)
(DNA; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT PCR (polymerase chain reaction)
Protein sequences
(PCR amplification of template DNA for **cell-free protein synthesis** system)

IT **Proteins**
Ras **proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(PCR amplification of template DNA for **cell-free protein synthesis** system)

IT Primers (nucleic acid)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(PCR amplification of template DNA for **cell-free protein synthesis** system)

IT **Translation, genetic**
(**cell-free**; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(double-stranded; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT Peptides, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(histidine-contg., use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(single-stranded; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT Peptides, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(streptavidin-binding, use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT Peptides, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(tag; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(terminator; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT Thioredoxins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT 9040-07-7P, Chloramphenicol acetyltransferase
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(PCR amplification of template DNA for **cell-free protein synthesis** system)

IT 140295-47-2, GenBank M21532 140531-55-1, GenBank M16206 142318-93-2, GenBank X65627 150947-97-0, GenBank L16904 175301-96-9, GenBank U51204 180770-00-7, GenBank D87663 186016-45-5, GenBank U85511 252790-02-6, GenBank S68022 384441-00-3, GenBank X13605 391530-17-9, GenBank M32599
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(PCR amplification of template DNA for **cell-free protein synthesis** system)

IT 484053-89-6
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(amino acid sequence; PCR amplification of template DNA for **cell-free protein synthesis** system)

IT 484100-82-5 484100-83-6 484100-84-7 484100-85-8 484100-86-9
484100-87-0 484100-88-1 484100-89-2 484100-90-5 484100-91-6
484100-92-7 484100-93-8 484100-94-9 484100-95-0 484100-96-1
484100-97-2 484100-98-3 484100-99-4 484101-00-0 484101-01-1
484101-02-2 484101-03-3 484101-04-4
RL: PRP (Properties)
(unclaimed nucleotide sequence; pCR amplification of template DNA for **cell-free protein synthesis** system)

IT 50812-37-8, Glutathione S-transferase
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use as tag; PCR amplification of template DNA for **cell-free protein synthesis** system)

L60 ANSWER 12 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:11293 HCAPLUS
DN 138:299991
TI Selenomethionine incorporation into a **protein** by **cell-free synthesis**
AU **Kigawa, Takanori**; Yamaguchi-Nunokawa, Emi; Kodama, Koichiro; Matsuda, Takayoshi; Yabuki, Takashi; Matsuda, Natsuko; Ishitani, Ryuichiro; Nureki, Osamu; **Yokoyama, Shigeyuki**
CS RIKEN Genomic Sciences Center, Yokohama, 230-0045, Japan
SO Journal of Structural and Functional Genomics (2002), 2(1), 29-35
CODEN: JSFGAW; ISSN: 1345-711X
PB Kluwer Academic Publishers
DT Journal
LA English
CC **9-5 (Biochemical Methods)**
Section cross-reference(s): 6
AB Multi-wavelength anomalous diffraction phasing is esp. useful for high-throughput structure detns. Selenomethionine substituted **proteins** are commonly used for this purpose. However, the cytotoxicity of selenomethionine drastically reduces the efficiency of its incorporation in in vivo **expression** systems. In the present study, an improved **E. coli cell-free**

protein synthesis system was used to incorporate selenomethionine into a **protein**, so that highly efficient incorporation could be achieved. A milligram quantity of selenomethionine-contg. Ras was obtained using the **cell-free** system with dialysis. The mass spectrometry anal. showed that more than 95% of the methionine residues were substituted with selenomethionine. The crystal of this **protein** grew under the same conditions and had the same unit **cell** consts. as those of the native Ras **protein**. The three-dimensional structure of this **protein**, detd. by multi-wavelength anomalous diffraction phasing, was almost the same as that of the Ras **protein** prepd. by in vivo **expression**. Therefore, the **cell-free synthesis** system could become a powerful **protein expression** method for high-throughput structure detns. by x-ray crystallog.

ST selenomethionine **protein** cell **synthesis**

IT Laser ionization mass spectrometry
(photodesorption, matrix-assisted; selenomethionine incorporation into **protein** by **cell-free synthesis**)

IT Laser desorption mass spectrometry
(photoionization, matrix-assisted; selenomethionine incorporation into **protein** by **cell-free synthesis**)

IT **Conformation**
(**protein**; selenomethionine incorporation into **protein** by **cell-free synthesis**)

IT Ras **proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(selenomethionine contg.; selenomethionine incorporation into **protein** by **cell-free synthesis**)

IT **Escherichia coli**
Time-of-flight mass spectrometry
(selenomethionine incorporation into **protein** by **cell-free synthesis**)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(selenomethionine incorporation into **protein** by **cell-free synthesis**)

IT Crystallography
(x-ray; selenomethionine incorporation into **protein** by **cell-free synthesis**)

IT 1464-42-2, Selenomethionine
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(selenomethionine incorporation into **protein** by **cell-free synthesis**)

RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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L60 ANSWER 13 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN

AN 2002:946589 HCAPLUS
DN 138:21811
TI Method for analyzing **protein** structure by mass spectrometry
IN Shen, Xinchun; **Kigawa, Takanori; Yokoyama, Shigeyuki**
PA Riken Corp., Japan
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DT **Patent**
LA Japanese
IC ICM G01N033-68
ICS G01N027-62; G01N033-53
CC **9-16 (Biochemical Methods)**
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002099437	A1	20021212	WO 2002-JP4473	20020508
	W: CA, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
	JP 2002372517	A2	20021226	JP 2001-164819	20010531
PRAI	JP 2001-164819	A	20010531		

AB A method is provided for analyzing **protein** mol. wt. by mass spectrometry. The method comprises: (a) a step for **synthesizing** a **protein fused** with a tag sequence with a **cell-free protein synthesis** system; (b) a step for bringing the **fused protein synthesized** into contact with a carrier capable of specifically binding to the tag sequence; (c) a step for recovering of the carrier bound to the **fused protein**; and (d) a step for measuring the mol. wt. of the **fused protein** by mass spectrometry (e.g., MALDI-TOF) using the **fused protein** bound to the carrier recovered. By using this method, a series of procedures of **synthesizing**, purifying and analyzing a **protein** can be efficiently and quickly carried out.

ST **protein** tag sequence carrier mass spectrometry

IT **Translation, genetic**
(**cell-free**; method for analyzing **protein** structure by mass spectroscopy)

IT **Proteins**
RL: NUU (Other use, unclassified); USES (Uses)
(ligand-binding; method for analyzing **protein** structure by mass spectroscopy)

IT Carriers
Mass spectrometry
Molecular weight

Protein motifs
(method for analyzing **protein** structure by mass spectroscopy)
IT **Proteins**
RL: ANT (Analyte); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study)
(method for analyzing **protein** structure by mass spectroscopy)
IT Amino acids, uses
Antibodies
Heterocyclic compounds
Metals, uses
Oligonucleotides
Peptides, uses
Receptors
RL: NUU (Other use, unclassified); USES (Uses)
(method for analyzing **protein** structure by mass spectroscopy)
IT Laser ionization mass spectrometry
(photodesorption, matrix-assisted, TOF; method for analyzing **protein** structure by mass spectroscopy)
IT Laser desorption mass spectrometry
(photoionization, matrix-assisted, TOF; method for analyzing **protein** structure by mass spectroscopy)
IT 67663-36-9, Ras
RL: ANT (Analyte); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study)
(**fused** with a histidine tag; method for analyzing **protein** structure by mass spectroscopy)
IT 9001-92-7, Proteinase
RL: NUU (Other use, unclassified); USES (Uses)
(method for analyzing **protein** structure by mass spectroscopy)
IT 9012-36-6, Agarose
RL: NUU (Other use, unclassified); USES (Uses)
(nickel-bound beads; method for analyzing **protein** structure by mass spectroscopy)
IT 478220-36-9
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(unclaimed amino acid sequence)
IT 478220-37-0
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(unclaimed nucleotide sequence)
IT 478235-80-2 478235-82-4 478235-84-6 478235-85-7, 8: PN: WO02099437 SEQID: 4 unclaimed DNA 478237-90-0, 9: PN: WO02099437 SEQID: 5 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; method for analyzing **protein** structure by mass spectrometry)
IT 478235-81-3 478235-83-5
RL: PRP (Properties)
(unclaimed **protein** sequence; method for analyzing **protein** structure by mass spectrometry)
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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(4) Pharmacia Biosensor Ab; JP 11-512518 A 1999
(5) Pharmacia Biosensor Ab; US 5955729 A 1999 HCAPLUS
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(18) Sequenom Inc; WO 9912040 A 1999 HCAPLUS

L60 ANSWER 14 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:869077 HCAPLUS
DN 137:365990
TI Detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system
IN Tajima, Kaori; **Kigawa, Takanori**; Shirouzu, Mikako; Yabuki, Takashi; Ishihara, Goushi; **Yokoyama, Shigeyuki**
PA Riken Corp., Japan
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DT **Patent**
LA Japanese
IC ICM C12N015-09
ICS C12P021-02; C12N001-21; C07K001-02
CC **9-14 (Biochemical Methods)**
Section cross-reference(s): 3, 6
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002090537	A1	20021114	WO 2002-JP4204	20020426
W: CA, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
JP 2003018999	A2	20030121	JP 2001-285145	20010919
PRAI JP 2001-135111	A	20010502		
JP 2001-285145	A	20010919		

AB In a process for producing a **protein** using a **cell-free protein synthesis** system, the system contains a detergent so that the **protein** can be **synthesized** without aggregation and denaturation. The above-described **protein** is a **protein** having a hydrophobic **domain** at least a part thereof, for example, a membrane **protein** or its fragment. The concn. of digitonin and Brij35 was 1-2 % and 0.01-0.5 %, resp. in the reaction buffer. The above-described detergent is a mild detergent which would not denature the **protein**, for example, a nonionic or amphoteric detergent.
ST detergent membrane **protein synthesis cell free** system
IT Polyoxyalkylenes, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(alkyl ethers; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)
IT Detergents
(ampholytic; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)
IT **Proteins**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(channel, **synthesis** of; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)
IT **Proteins**

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(hydrophobic, **synthesis** of; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT DNA
Lipids, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(in reaction buffer; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(membrane, hydrophobic, **synthesis** of; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT Detergents
(nonionic; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT **Translation, genetic**
(of membrane **protein**, method of; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT Aggregation
(of **protein**, prevention of; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT Denaturation
(prevention of; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT Receptors
Transport **proteins**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(**synthesis** of; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT 2605-79-0, N,N-Dimethyldecylamine N-oxide 7305-30-8 9002-92-0, Brij35 9005-63-4, Polyoxyethylene sorbitan 11024-24-1, Digitonin 15163-36-7 19327-40-3, 3,6,9,12,15-Pentaoxatricosan-1-ol 25322-68-3D, alkyl ethers 29836-26-8, Octyl glucoside 31835-06-0, Sucrose mono decanoate 69227-93-6, .beta.-Dodecyl maltoside 75621-03-3, Chaps 85261-19-4, Nonanoyl-N-methyl glucamide 85261-20-7, Decanoyl-N-methyl glucamide 85618-20-8, .beta.-Heptylthioglucoside 85618-21-9, .beta.-Octylthioglucoside
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

IT 475460-14-1, 1: PN: WO02090537 SEQID: 1 unclaimed DNA 475460-15-2 475460-16-3, 3: PN: WO02090537 SEQID: 3 unclaimed DNA 475460-17-4, 4: PN: WO02090537 SEQID: 4 unclaimed DNA 475460-18-5 475460-19-6 475460-20-9, 7: PN: WO02090537 SEQID: 7 unclaimed DNA 475460-21-0, 8: PN: WO02090537 SEQID: 8 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; detergents used for biosynthesis of membrane **protein** in **cell-free protein synthesis** system)

RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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(9) Rikagaku Kabushiki Kaisha; JP 2000175695 A 2000 HCAPLUS
(10) Wakenyaku Co Ltd; WO 0068412 A1 2000 HCAPLUS
(11) Wakenyaku Co Ltd; EP 1176210 A1 2000 HCAPLUS
(12) Wakenyaku Co Ltd; JP 2000316594 A 2000 HCAPLUS

L60 ANSWER 15 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:704583 HCAPLUS
DN 137:227659
TI **Cell-free synthesis** of **soluble protein domains** for **three-dimensional** structure analysis
IN **Seki, Eiko; Kikawa, Takanori; Yokoyama, Shigeyuki**
PA Institute of Physical and Chemical Research, Japan
SO Jpn. Kokai Tokkyo Koho, 9 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
IC ICM C12N015-09
ICS C07K014-00; C07K019-00; C12N001-21; C12P021-02; G01N023-20; G01N024-08; G01N033-68; C12R001-19
CC **3-2** (Biochemical Genetics)
Section cross-reference(s): **9**
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 2002262873	A2	20020917	JP 2001-62703	20010306
	US 2002142387	A1	20021003	US 2001-994573	20011126 <-- Applicant
PRAI	JP 2001-62703	A	20010306		

AB A method for **cell-free synthesis** of **sol. protein domains** suitable for **three-dimensional** structure anal., is disclosed. **Protein domains** are **expressed** as **fusion proteins** with a detectable **protein** such as **enzyme**, binding **protein**, luminescent **protein**, or fluorescent **protein**, and selected. They can be used for structure detn. by x-ray crystallog. or NMR. **Expression** of mammalian growth hormone receptor binding **protein** Grb2 **domains** as **fusion protein** with **GFP** mutant is described. Fragments having **domain** structure at the N-terminal produced stronger fluorescence than those having **domain** structure in the middle.
ST **cell free synthesis protein domain three dimensional** structure analysis
IT **Proteins**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(Grb-2, **domain expression** of; **cell-free synthesis** of **sol. protein domains** for **three-dimensional** structure anal.)
IT **Molecular structure determination methods**
(NMR spectrometric; **cell-free synthesis** of **sol. protein domains** for **three-dimensional** structure anal.)
IT **Crystal structure determination methods**

Protein motifs
(**cell-free synthesis** of **sol.**
protein domains for **three-**
dimensional structure anal.)

IT **Fusion proteins (chimeric proteins)**
RL: ARU (Analytical role, unclassified); BPN (Biosynthetic preparation); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**cell-free synthesis** of **sol.**
protein domains for **three-**
dimensional structure anal.)

IT **Translation, genetic**
(**cell-free; cell-free**
synthesis of **sol. protein domains**
for **three-dimensional** structure anal.)

IT **Proteins**
RL: ARU (Analytical role, unclassified); BPN (Biosynthetic preparation); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**green fluorescent, fusion**
protein of; **cell-free synthesis**
of **sol. protein domains** for **three**
-dimensional structure anal.)

IT **Conformation**
Quaternary structure
Tertiary structure
(**protein; cell-free synthesis**
of **sol. protein domains** for **three**
-dimensional structure anal.)

IT **Molecular structure determination methods**
(x-ray diffractometric; **cell-free synthesis**
of **sol. protein domains** for **three**
-dimensional structure anal.)

IT 459469-42-2
RL: PRP (Properties)
(unclaimed nucleotide sequence; **cell-free**
synthesis of **sol. protein domains**
for **three-dimensional** structure anal.)

L60 ANSWER 16 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:704578 HCAPLUS
DN 137:212639
TI **Cell-free synthesis** of heavy atom-containing
proteins for x-ray crystallography structural analysis
IN Nunokawa, Emi; **Kikawa, Takanori**; Yabuki, Takashi; **Yokoyama, Shigeyuki**
PA Institute of Physical and Chemical Research, Japan
SO Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
IC ICM C12N015-09
ICS C07K001-34; C12P021-00; C12N001-16; C12N001-20
CC 6-3 (General Biochemistry)
Section cross-reference(s): **9**, 75
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 2002262867	A2	20020917	JP 2001-65799	20010308
	US 2002168705	A1	20021114	US 2001-989974	20011120
PRAI	JP 2001-65799	A	20010308		

AB A method for large-scale **cell-free synthesis** of heavy atom-contg. **proteins** suitable for x-ray crystallog.

structural anal. using dialysis, is disclosed. **Cell** ext. of **E. coli**, hyperthermophilic archaeon, or yeast, is used. It also includes ATP regeneration system, macromol. adsorbent, and reducing agent. Creatine kinase and creatine phosphate are used for ATP regeneration. Amino acids contg. mercury, platinum, iodine, iron, or selenium, such as selenocysteine or selenomethionine, are to be incorporated. **Synthesis** of selenomethionine-contg. Ras **protein** by **cell-free synthesis** system, crystn. by hanging-drop vapor-diffusion method, and structural anal. by multiwavelength anomalous diffraction (MAD), are described. The three dimensional structure model produced was identical to those of unlabeled **proteins** produced in vivo and in **cell-free** system.

ST **cell free protein synthesis** heavy atom replacement xray crystallog

IT Archaebacteria (Archaea)
Escherichia coli
Yeast
(**cell** ext. of; **cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT **Crystal structure determination methods**
Dialysis
Reducing agents
(**cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT Ras **proteins**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(**cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT **Translation, genetic**
(**cell-free; cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT Amino acids, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(heavy atom-contg.; **cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT **Conformation**
Quaternary structure
Tertiary structure
(**protein; cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT **Molecular structure determination methods**
(x-ray diffractometric; **cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT 7439-89-6, Iron, biological studies 7439-97-6, Mercury, biological studies 7440-06-4, Platinum, biological studies 7553-56-2, Iodine, biological studies 7782-49-2, Selenium, biological studies
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(amino acids contg.; **cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT 3211-76-5, Selenomethionine 3614-08-2, Selenocysteine
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(**cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT 56-65-5, 5'-ATP, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(regeneration system for; **cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT 67-07-2, Creatine phosphate
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use for ATP regeneration; **cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

IT 9001-15-4, Creatine kinase
RL: BUU (Biological use, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses)
(use for ATP regeneration; **cell-free synthesis** of heavy atom-contg. **proteins** for x-ray crystallog. structural anal.)

L60 ANSWER 17 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:576300 HCAPLUS
DN 137:258909
TI An engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of an unnatural amino acid into **proteins** in eukaryotic translation and its application in a wheat germ **cell-free** system
AU Kiga, Daisuke; Sakamoto, Kensaku; Kodama, Koichiro; **Kigawa, Takanori**; Matsuda, Takayoshi; Yabuki, Takashi; Shirouzu, Mikako; Harada, Yoko; Nakayama, Hiroshi; Takio, Koji; Hasegawa, Yoshinori; Endo, Yaeta; Hirao, Ichiro; **Yokoyama, Shigeyuki**
CS RIKEN Genomic Sciences Center, Yokohama, 230-0045, Japan
SO Proceedings of the National Academy of Sciences of the United States of America (2002), 99(15), 9715-9723
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
CC 6-1 (General Biochemistry)
Section cross-reference(s): 7
AB Tyrosyl-tRNA **synthetase** (TyrRS) from **Escherichia coli** was engineered to preferentially recognize 3-iodo-L-tyrosine rather than L-tyrosine for the site-specific incorporation of 3-iodo-L-tyrosine into **proteins** in eukaryotic translation systems. The wild-type TyrRS does not recognize 3-iodo-L-tyrosine, because of the bulky iodine substitution. On the basis of the reported crystal structure of Bacillus stearothermophilus TyrRS, three residues, Y37, Q179, and Q195, in the L-tyrosine-binding site were chosen for mutagenesis. Thirty-four single amino acid replacements and 16 of their combinations were screened by in vitro biochem. assays. A combination of the Y37V and Q195C mutations changed the amino acid specificity in such a way that the variant TyrRS activates 3-iodo-L-tyrosine 10-**fold** more efficiently than L-tyrosine. This engineered **enzyme**, TyrRS(V37C195), was tested for use in the wheat germ **cell-free** translation system, which has recently been significantly improved, and is now as productive as conventional **recombinant** systems. During the translation in the wheat germ system, an **E. coli** suppressor tRNATyr was not aminoacylated by the wheat germ **enzymes**, but was aminoacylated by the **E. coli** TyrRS(V37C195) variant with 3-iodo-L-tyrosine. After the use of the 3-iodotyrosyl-tRNA in translation, the resultant uncharged tRNA could be aminoacylated again in the system. A mass spectrometric anal. of the

produced **protein** revealed that more than 95% of the amino acids incorporated for an amber codon were iodotyrosine, whose concn. was only twice that of L-tyrosine in the translation. Therefore, the variant **enzyme**, 3-iodo-L-tyrosine, and the suppressor tRNA can serve as an addnl. set orthogonal to the 20 endogenous sets in eukaryotic in vitro translation systems.

ST engineering Escherichia tyrosyl tRNA **synthetase** unnatural amino acid translation

IT Codons
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(amber; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT **Enzyme** kinetics
Protein engineering
Translation, genetic
(engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT **Enzyme functional** sites
(substrate-binding, tyrosine-binding; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT tRNA
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(suppressor; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT 9023-45-4P, Tyrosyl-tRNA **synthetase**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT 60-18-4, L-Tyrosine, biological studies 70-78-0, 3-Iodo-L-tyrosine
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT 52-90-4, L-Cysteine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(residue no. 195 in mutant **synthetase**; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT 56-85-9, L-Glutamine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(residue no. 195 in wild-type **synthetase**; engineered **Escherichia coli** tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

IT 72-18-4, L-Valine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(residue no. 37 in mutant **synthetase**; engineered

Escherichia coli tyrosyl-tRNA **synthetase** for site-specific incorporation of unnatural amino acid into **proteins** in eukaryotic translation and application in a wheat germ **cell-free** system)

RE.CNT 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L60 ANSWER 18 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN

AN **2002:504945** HCAPLUS

DN **137:62163**

TI Preparation of **recombinant protein** as chaperon **fusion protein**

IN Furutani, Masahiro; Hata, Junichi; Togi, Akiko

PA Sekisui Chemical Co., Ltd., Japan; Marine Biotechnology Institute Co., Ltd.

SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2

DT Patent

LA Japanese
IC ICM C12P021-02
ICS C12N015-09; C07K019-00
CC 15-2 (Immunochemistry)
Section cross-reference(s): **3**
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002052029	A1	20020704	WO 2001-JP11438	20011226
	WO 2002052029	C2	20030424		

W: AU, CA, JP, KR, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

PRAI JP 2000-395740 A 20001226

AB Provided is an **expression** and **cell-free translation** system for a **recombinant protein** as **fusion protein** with chaperonin subunit. A target **protein** is incorporated into the tertiary structure of chaperonin as a **fusion protein** with a chaperonin subunit (namely, a mol. chaperonin of about 60 kDa, heat shock **protein** of 60 kDa or thermosome) to thereby inhibit the **expression** of toxicity of the target **protein** in the host,. **Digestion** with a protease or CNBr at a methionine residue can be used to cleave off the target **protein** from the chaperonin subunit. Bacteria, archaea, or eukaryote derived chaperonin is used. Bacteria, yeast, animal **cell**, plant **cell**, insect **cell** can be used as host. Mammalian antibody heavy chain, light chain, single chain antibody Fv region, virus antigen, transmembrane receptor, or cytokine may be the target **protein**. **Expression** of hepatitis virus B surface antigen, hepatitis virus C core antigen, mouse anti-chicken lysozyme single chain antibody, human antibody heavy chain const. region, human interferon .alpha.2b, or human serotonin receptor as **fusion protein** with chaperonin GroEL, is described.

ST **protein recombinant expression** chaperonin **fusion**

IT Chemokine receptors
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(CXCR4; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Chaperonins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(GroEL; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Animal cell
Bacteria (Eubacteria)
Escherichia coli
Insecta
Plant cell
Yeast
(as host; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Archaebacteria (Archaea)
Eukaryota
(chaperonin from; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Immunoglobulins
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(fragments, Fv region; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Immunoglobulins

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(heavy chains; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Antigens
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(hepatitis B core; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Immunoglobulins
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(light chains; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT 5-HT receptors
Antibodies
Antigens
Cytokines
Fusion proteins (chimeric proteins)
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Chaperonins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Heat-shock **proteins**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Antibodies
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(single chain; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Antigens
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(surface; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT Interferons
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(.alpha.-2b; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT 439059-34-4, 1: PN: WO02052029 SEQID: 1 unclaimed DNA 439059-35-5, 2: PN: WO02052029 SEQID: 3 unclaimed DNA 439059-36-6, 3: PN: WO02052029 SEQID: 4 unclaimed DNA 439059-37-7, 4: PN: WO02052029 SEQID: 5 unclaimed DNA 439059-38-8, 5: PN: WO02052029 SEQID: 6 unclaimed DNA 439059-39-9, 6: PN: WO02052029 SEQID: 7 unclaimed DNA 439059-40-2, 7: PN: WO02052029 SEQID: 8 unclaimed DNA 439059-41-3, 8: PN: WO02052029 SEQID: 2 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT 9001-92-7, Proteinase
RL: BUU (Biological use, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses)
(use in cleavage; prepn. of **recombinant protein** as chaperon **fusion protein**)

IT 506-68-3, Cyanogen bromide ((CN)Br)
RL: RCT (Reactant); RACT (Reactant or reagent)
(use in cleavage; prepn. of **recombinant protein** as chaperon **fusion protein**)
RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L60 ANSWER 19 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:442973 HCAPLUS
DN 137:29466
TI High-throughput **cell-free protein expression** system for structural genomics and proteomics studies
AU **Kigawa, Takanori; Yokoyama, Shigeyuki**
CS Genomic Sci. Cent., RIKEN, Japan
SO Tanpakushitsu Kakusan Koso (2002), 47(8, Zokan), 1014-1019
CODEN: TAKKAJ; ISSN: 0039-9450
PB Kyoritsu Shuppan
DT Journal; General Review
LA Japanese
CC 6-0 (General Biochemistry)
Section cross-reference(s): **9**
AB A review on tech. development of high-throughput **protein expression** using **cell-free protein synthesis** system and its application to structure proteomics studies.
ST review structure proteomics **protein expression** system
IT **Translation, genetic**
(**cell-free**; high-throughput **cell-free protein expression** system for structural genomics and proteomics studies)
IT **Proteins**
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(high-throughput **cell-free protein expression** system for structural genomics and proteomics studies)
IT Genome
(structural genomics; high-throughput **cell-free protein expression** system for structural genomics and proteomics studies)
IT Proteome
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(structure proteomics; high-throughput **cell-free protein expression** system for structural genomics and proteomics studies)

L60 ANSWER 20 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:235434 HCAPLUS
DN 137:164318
TI **Expression** of FLAG **Fusion Proteins** in Insect Cells: Application to the Multi-subunit Transcription/DNA Repair Factor TFIIH
AU Jawhari, Anass; Uhring, Muriel; Crucifix, Corinne; Fribourg, Sebastien; Schultz, Patrick; Poterszman, Arnaud; Egly, Jean Marc; Moras, Dino
CS Institut de Genetique et de Biologie Cellulaire et Moleculaire, CNRS/INSERM/ULP, College de France, Illkirch, 67404, Fr.
SO Protein Expression and Purification (2002), 24(3), 513-523

CODEN: PEXPEJ; ISSN: 1046-5928
PB Elsevier Science
DT Journal
LA English
CC **3-2** (Biochemical Genetics)
Section cross-reference(s): 12, 13
AB The multi-subunit transcription/DNA repair factor TFIIH was used as a model system to show that the **expression** of FLAG **fusion proteins** in insect cells constitutes a versatile tool for both structural and **functional** investigations. In the present study, we have constructed **recombinant** baculoviruses **expressing** the four core TFIIH subunits **fused** at their N-terminus to the FLAG peptide. Using these **recombinant** viruses we have established protocols based on anti-FLAG immunoaffinity chromatog. that allow the systematic anal. of pairwise interaction within multiprotein complexes and have developed a double tag strategy (FLAG and hexahistidine tags) for the identification and purifn. of stable TFIIH subcomplexes. A simple purifn. procedure was developed that leads to the isolation of **recombinant** TFIIH contg. the full set of subunits. The purified **recombinant** TFIIH was shown to be active in a transcription assay and to be structurally homologous to the endogenous complex by electron microscopy and image anal.
ST human multisubunit TFIIH **expression** purifn baculovirus **vector** Sf9 cell
IT **Enzymes**, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(DNA helicase, XPB, His-tagged TFIIH subunit; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)
IT Animal cell line
(SF9, infection; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)
IT Transcription factors
RL: PUR (Purification or recovery); PREP (Preparation)
(TFIIH (transcription factor IIH); **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)
IT Transcription, genetic
(**cell-free**; **expression** of FLAG **fusion proteins** in insect **cells**, application to the human multi-subunit transcription/DNA repair factor TFIIH)
IT Autographa californica nucleopolyhedrovirus
Human
Molecular **cloning**
(**expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)
IT **Fusion proteins (chimeric proteins)**
RL: BPN (Biosynthetic preparation); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(**expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)
IT Molecular association
(of TFIIH subunits; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)
IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(p34, FLAG-tagged TFIIH subunit; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)

IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(p44, FLAG-tagged TFIIH subunit; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)

IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(p52, FLAG-tagged TFIIH subunit; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)

IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(p62, FLAG-tagged TFIIH subunit; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)

IT Viral **vectors**
(pSK277; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)

IT Simulation and Modeling, physicochemical
(**three-dimensional**; **expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)

IT 64134-30-1, Hexahistidine 98849-88-8, FLAG peptide
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**expression** of FLAG **fusion proteins** in insect cells, application to the human multi-subunit transcription/DNA repair factor TFIIH)

RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L60 ANSWER 21 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:123933 HCAPLUS
DN 137:88980
TI An unnatural base pair for incorporating amino acid analogs into **proteins**
AU Hirao, Ichiro; Ohtsuki, Takashi; Fujiwara, Tsuyoshi; Mitsui, Tsuneo;

Yokogawa, Tomoko; Okuni, Taeko; Nakayama, Hiroshi; Takio, Koji; Yabuki, Takashi; **Kigawa, Takanori**; Kodama, Koichiro; Yokogawa, Takashi; Nishikawa, Kazuya; **Yokoyama, Shigeyuki**
CS Yokoyama Cytologic Project, ERATO, JST, c/o RIKEN, Wako-shi, Saitama, 351-0198, Japan
SO Nature Biotechnology (2002), 20(2), 177-182
CODEN: NABIF9; ISSN: 1087-0156
PB Nature America Inc.
DT Journal
LA English
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): **6**, 9
AB An unnatural base pair of 2-amino-6-(2-thienyl)purine (denoted by s) and pyridin-2-one (denoted by y) was developed to expand the genetic code. The ribonucleoside triphosphate of y was site-specifically incorporated into RNA, opposite s in a template, by T7 RNA polymerase. This transcription was coupled with translation in an **Escherichia coli cell-free** system. The yAG codon in the transcribed ras mRNA was recognized by the CUs anticodon of a yeast tyrosine tRNA (tRNA) variant, which had been **enzymically** aminoacylated with an unnatural amino acid, 3-chlorotyrosine. Site-specific incorporation of 3-chlorotyrosine into the Ras **protein** was demonstrated by liq. chromatog.-mass spectrometry (LC-MS) anal. of the products. This coupled transcription-translation system will permit the efficient **synthesis** of **proteins** with a tyrosine analog at the desired position.
ST **protein** engineering chlorotyrosine genetic translation unnatural base pair; tyrosine tRNA RNA sequence human gene cHa ras **protein**
IT Amino acids, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(analogs; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)
IT Nucleic acid bases
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(analogs; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)
IT Gene, animal
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(c-Ha-ras, mRNA, yAG codon-contg.; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)
IT Transcription, genetic
Translation, genetic
(**cell-free**; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)
IT DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(double-stranded, template, s .cntdot. T base pair; unnatural base pair of 2-amino-6-(2-thienyl)purine (denoted s) and pyridin-2-one (denoted y) for incorporating amino acid analogs into **proteins** by a coupled **cell-free** transcription-translation reaction)
IT **mRNA sequences**
(of human c-Ha-ras with incorporated pyridin-2-one (y))
IT Ras **proteins**
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological

study); PREP (Preparation)
(p21c-Ha-ras, 3-chlorotyrosine-modified; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT Nucleic acid bases
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(pairing; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT Anticodons
RL: BUU (Biological use, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**synthetic**, CUs; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT Codons
RL: BUU (Biological use, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**synthetic**, yAG; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT tRNA
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); PRP (Properties); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(tyrosine-specific, CUs anticodon-contg.; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT Human
Protein engineering
(unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT RNA
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT 440200-36-2P
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(amino acid sequence; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT 439618-75-4P
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); PRP (Properties); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(nucleotide sequence; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT 439618-71-0P
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(nucleotide sequence; unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with 3-chlorotyrosine at position 32)

IT 7423-93-0P
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(unnatural base pair for incorporating amino acid analogs into **proteins**, prodn. of human Ras **protein** with

3-chlorotyrosine at position 32)
IT 142-08-5, Pyridin-2-one 156489-35-9 382146-88-5
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(unnatural base pair of 2-amino-6-(2-thienyl)purine (denoted s) and pyridin-2-one (denoted y) for incorporating amino acid analogs into **proteins** by a coupled **cell-free** transcription-translation reaction)
RE.CNT 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L60 ANSWER 22 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2002:47341** HCAPLUS
DN **137:104208**
TI The many faces of **protein expression**
AU Janssen, Deborah
CS USA
SO Genomics & Proteomics (2001), 1(3), 53-57
CODEN: GPERA6; ISSN: 1536-7495
PB Cahners Business Information
DT Journal; General Review
LA English
CC **3-0** (Biochemical Genetics)
Section cross-reference(s): 6, 16
AB A review. Various **protein expression** systems that provide easy, rapid, accurate **protein** characterization for a wide range of user application have been developed. One such system is called pET (plasmid for **expression** by T7 RNA polymerase) **expression** system, developed by Novagen Inc., for eukaryotic genes in **E. coli**. The Novagen pET **expression** system offers 39 different pET **vectors**, 36 different **expression** strains, 14 **fusion** tag choices, and many options for rapid anal. and purifn. of target **proteins**. Another system is the Rapid **Translation** System (RTS), a **cell-free protein expression** platform, which eliminates the need for housekeeping cellular activities.
ST review **protein expression** system bacterium eukaryote Escherichia
IT **Escherichia coli**
(**expression** host; com. **expression** systems for manuf. of **proteins** in prokaryotic and eukaryotic systems)
IT **Translation, genetic**
(in vitro, for **protein** manuf.; com. **expression** systems for manuf. of **proteins** in prokaryotic and eukaryotic systems)
IT Plasmid **vectors**
(pET; com. **expression** systems for manuf. of **proteins** in prokaryotic and eukaryotic systems)
IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(prepn. by **translation** of; com. **expression** systems for manuf. of **proteins** in prokaryotic and eukaryotic systems)
IT Fermentation
(**protein**; com. **expression** systems for manuf. of **proteins** in prokaryotic and eukaryotic systems)

L60 ANSWER 23 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2001:586343** HCAPLUS
DN **136:196343**
TI The **cell-free protein** biosynthesis - applications and analysis of the system
AU Lamla, Thorsten; Mammeri, Kerstin; Erdmann, Volker A.
CS Institut fur Biochemie, Freie Universitat Berlin, Berlin, D-14195, Germany
SO Acta Biochimica Polonica (2001), 48(2), 453-465
CODEN: ABPLAF; ISSN: 0001-527X
PB Polish Biochemical Society
DT Journal
LA English
CC **9-2** (**Biochemical Methods**)
Section cross-reference(s): 6
AB The in vitro **protein** biosynthesis has the potentials to become a powerful technol. for biochem. research. Beside the detn. of structure and **function** the in vitro evolution of **proteins** is

also of great interest. The system described was used to produce bovine heart fatty acid binding **protein** (FABP) and bacterial chloramphenicol acetyltransferase (CAT) with and without **fusion** of the Strep-tag II affinity peptide. The **proteins** were purified after and during **protein** biosynthesis by using a StrepTactin Sepharose matrix. No significant influence of the Strep-tag and the conditions during the affinity chromatog. on maturation or activity of the **protein** was obsd. The in vitro evolution of **proteins** is feasible by means of ribosome display. The selection of a specific mRNA coding for a shortened FABP with a N-terminal His-tag via the accompanying **protein** property was shown. Goal of the selection was to bind the FABP via the His-tag on Ni(II)-IDA-agarose. After nine cycles of transcription, translation, affinity selection and RT-PCR the **protein** with the His-tag could be enriched 108-**fold**. In order to correlate a possible relationship between changes in **protein** population and biol. **function** studies were initiated in which 2-dimensional **protein** patterns of the total in vitro system were compared after 0 and 2 h reaction time. The very interesting findings are that a no. of **proteins** disappear, while others are newly formed during **protein synthesis**.

ST **cell free** system **protein** biosynthesis; fatty acid binding **protein** biosynthesis; chloramphenicol acetyltransferase biosynthesis

IT **Proteins**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(FABP (fatty acid-binding **protein**); **cell-free protein** biosynthesis - applications and anal. of system)

IT **Translation, genetic**
(**cell-free protein** biosynthesis - applications and anal. of system)

IT mRNA
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**cell-free protein** biosynthesis - applications and anal. of system)

IT Gel electrophoresis
(two-dimensional; **cell-free protein** biosynthesis - applications and anal. of system)

IT 9040-07-7P, Chloramphenicol acetyltransferase
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(**cell-free protein** biosynthesis - applications and anal. of system)

RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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L60 ANSWER 24 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2001:232988** HCAPLUS
DN **135:41519**
TI **Cell-Free** Production of Biologically Active Polypeptides: Application to the **Synthesis** of Antibacterial Peptide Cecropin
AU Martemyanov, Kirill A.; Shirokov, Vladimir A.; Kurnasov, Oleg V.; Gudkov, Anatoly T.; Spirin, Alexander S.
CS Institute of Protein Research, Russian Academy of Sciences, Pushchino, Moscow Region, 142292, Russia
SO Protein Expression and Purification (2001), 21(3), 456-461
CODEN: PEXPEJ; ISSN: 1046-5928
PB Academic Press
DT Journal
LA English
CC **3-1** (Biochemical Genetics)
Section cross-reference(s): 6
AB An approach to preparative prodn. of polypeptides, including uneasily testable, degradable, and toxic ones, is proposed on the basis of in vitro **expression** systems of last generation, such as continuous-exchange **cell-free** and continuous-flow **cell-free** transcription-translation systems. The approach implies that a polypeptide of interest is **synthesized** as a **fusion protein** with the polypeptide linked to **green fluorescent protein** (**GFP**) through a cleavable spacer. The **GFP** moiety provides direct visualization and quant. monitoring of the polypeptide **synthesis**, as well as **soly**. and stability of the product. The **synthesis** of **functionally** active antibacterial polypeptide cecropin P1 (31 amino acid residues) has been demonstrated. (c) 2001 Academic Press.
ST toxic **protein fusion** product **cell free synthesis**; labile **protein fusion** product **cell free synthesis**; **green fluorescent protein fusion** product **cell free synthesis**; translation in vitro toxic labile **protein fusion** product; cecropin manuf **fusion** product **cell free** translation
IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(10, ribosome binding site of; **synthesis** of toxic or labile **proteins** as cleavable **fusion** products with **green fluorescent protein** in **cell-free** systems)
IT Transcription, genetic
(**cell-free**, translation coupled; **synthesis** of toxic or labile **proteins** as cleavable **fusion** products with **green fluorescent protein** in **cell-free** systems)
IT **Translation, genetic**
(**cell-free**; **synthesis** of toxic or labile

proteins as cleavable **fusion** products with **green fluorescent protein** in **cell** **-free** systems)

IT **Proteins, specific or class**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(**green fluorescent, fusion** **products; synthesis** of toxic or labile **proteins** as cleavable **fusion products** with **green fluorescent protein** in **cell** **-free** systems)

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(ribosome-binding site, gene 10, in transcription translation constructs; **synthesis** of toxic or labile **proteins** as cleavable **fusion** products with **green** **fluorescent protein** in **cell-free** systems)

IT Coliphage T7
(transcription-translation **vectors** derived from; **synthesis** of toxic or labile **proteins** as cleavable **fusion** products with **green fluorescent** **protein** in **cell-free** systems)

IT 506-68-3, Cyanogen bromide
RL: RCT (Reactant); RACT (Reactant or reagent)
(**fusion proteins** cleavable by; **synthesis** of toxic or labile **proteins** as cleavable **fusion** products with **green fluorescent protein** in **cell-free** systems)

IT 9014-74-8, Enterokinase
RL: CAT (Catalyst use); USES (Uses)
(**fusion proteins** contg. cleavage site for; **synthesis** of toxic or labile **proteins** as cleavable **fusion** products with **green fluorescent** **protein** in **cell-free** systems)

IT 80802-79-5DP, Cecropin, **fusion** products with **green fluorescent protein**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(**synthesis** of toxic or labile **proteins** as cleavable **fusion** products with **green fluorescent** **protein** in **cell-free** systems)

RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L60 ANSWER 25 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2001:152863** HCAPLUS
DN **134:204756**
TI Methods for the detection, analysis and isolation of nascent **proteins**
IN Rothschild, Kenneth J.; Gite, Sadanand; Olejnik, Jerzy
PA Ambergen, Inc., USA
SO PCT Int. Appl., 204 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12P021-06
ICS C12N009-00
CC **9-16** (**Biochemical Methods**)
Section cross-reference(s): 1, **3**
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001014578	A1	20010301	WO 2000-US23233	20000823
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6303337	B1	20011016	US 1999-382950	19990825
US 6306628	B1	20011023	US 1999-382736	19990825
EP 1210449	A1	20020605	EP 2000-957758	20000823
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
US 2002132248	A1	20020919	US 2001-973145	20011009
US 2003092031	A1	20030515	US 2002-174368	20020618
PRAI US 1999-382736	A	19990825		
US 1999-382950	A	19990825		
WO 2000-US23233	W	20000823		
US 2002-49332	A2	20020621		

AB This invention relates to non-radioactive markers that facilitate the detection and anal. of nascent **proteins translated** within cellular or **cell-free translation** systems. Nascent **proteins** contg. these markers can be rapidly and efficiently detected, isolated and analyzed without the handling and disposal problems assocd. with radioactive reagents. Preferred markers are dipyrromethene boron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes.

ST detection analysis isolation nascent **protein**
IT Codons
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(AUG; methods for detection, anal. and isolation of nascent **proteins**)

IT **Proteins**, specific or class
RL: ANT (Analyte); ANST (Analytical study)
(Immunogenic; methods for detection, anal. and isolation of nascent **proteins**)
IT **Proteins**, specific or class
RL: ANT (Analyte); ANST (Analytical study)
(**Recombinant**; methods for detection, anal. and isolation of nascent **proteins**)
IT Wheat
(germ; methods for detection, anal. and isolation of nascent **proteins**)
IT Transformation, neoplastic
(immortalization; methods for detection, anal. and isolation of nascent **proteins**)
IT tRNA
RL: BUU (Biological use, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent); USES (Uses)
(initiator; methods for detection, anal. and isolation of nascent **proteins**)
IT **Proteins**, specific or class
RL: ANT (Analyte); ANST (Analytical study)
(lipid-binding; methods for detection, anal. and isolation of nascent **proteins**)
IT Affinity
Aminoacylation
Animal tissue culture
Bacteria (Eubacteria)
Cell
Dialysis
Dog (Canis familiaris)
Drugs
Dyes
Escherichia coli
Flow
Fluorometry
Frog
Immunoassay
Insect (Insecta)
Mutation
Neoplasm
PCR (polymerase chain reaction)
Pancreas
Parasite
Rabbit
Reticulocyte
Temperature effects, biological
Test kits
Translation, genetic
Virus
(methods for detection, anal. and isolation of nascent **proteins**)
IT Carbohydrates, analysis
Cytokines
Enzymes, analysis
Fusion proteins (chimeric proteins)
Hormones, animal, analysis
Protein formation factors
RL: ANT (Analyte); ANST (Analytical study)
(methods for detection, anal. and isolation of nascent **proteins**)
IT Proteome
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)

(methods for detection, anal. and isolation of nascent **proteins**
)
IT Gene, animal
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT Primers (nucleic acid)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT tRNA
RL: BUU (Biological use, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(misaminoacylated; methods for detection, anal. and isolation of nascent **proteins**)
IT **Proteins**, general, analysis
RL: ANT (Analyte); PUR (Purification or recovery); ANST (Analytical study); PREP (Preparation)
(nascent; methods for detection, anal. and isolation of nascent **proteins**)
IT **Proteins**, specific or class
RL: ANT (Analyte); ANST (Analytical study)
(nucleic acid-binding; methods for detection, anal. and isolation of nascent **proteins**)
IT Egg
(oocyte; methods for detection, anal. and isolation of nascent **proteins**)
IT tRNA
RL: BUU (Biological use, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent); USES (Uses)
(suppressor; methods for detection, anal. and isolation of nascent **proteins**)
IT Hemolysins
RL: ANT (Analyte); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process)
(.alpha.-; methods for detection, anal. and isolation of nascent **proteins**)
IT 9001-92-7, Protease
RL: ANT (Analyte); ANST (Analytical study)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT 138026-71-8, Bodipy
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); RCT (Reactant); ANST (Analytical study); BIOL (Biological study); RACT (Reactant or reagent); USES (Uses)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT 91-64-5DP, Coumarin, derivs.
RL: ARG (Analytical reagent use); RCT (Reactant); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); RACT (Reactant or reagent); USES (Uses)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT 71-00-1, Histidine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT 60-32-2, 6-Aminocaproic acid 3992-42-5 35231-44-8 58178-21-5 69976-70-1 87424-19-9 105047-45-8
RL: RCT (Reactant); RACT (Reactant or reagent)
(methods for detection, anal. and isolation of nascent **proteins**

)
IT 58-61-7P, Adenosine, preparation 610-15-1P 6851-99-6P 35013-72-0P
72040-64-3P 87424-17-7P 110744-77-9P 133852-19-4P 133852-20-7P
174406-74-7P 208660-68-8P 250610-57-2P 328387-23-1P 328387-40-2P
328387-48-0P 328387-50-4P 328387-52-6P 328387-54-8P 328387-56-0P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT 133852-21-8P 328387-26-4P 328387-45-7DP, reaction with amino acids
328564-07-4P
RL: SPN (Synthetic preparation); PREP (Preparation)
(methods for detection, anal. and isolation of nascent **proteins**
)
IT 328601-46-3 328601-47-4, 2: PN: WO0114578 PAGE: 15 unclaimed RNA
328601-48-5, 3: PN: WO0114578 PAGE: 15 unclaimed RNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; methods for the detection, anal. and isolation of nascent **proteins**)
IT 64134-30-1 92000-76-5 98849-88-8 103425-05-4 112218-36-7
145646-22-6 169802-74-8 169802-75-9 174144-06-0 205938-74-5
245445-88-9 268741-28-2 328547-57-5 328547-58-6 328601-49-6
328601-50-9 328601-51-0 328601-52-1 328601-53-2 328601-54-3
328601-55-4 328601-56-5 328601-57-6 328601-58-7 328601-59-8
328601-60-1 328601-61-2 328601-62-3 328601-63-4 328601-64-5
328601-65-6 328601-66-7
RL: PRP (Properties)
(unclaimed sequence; methods for the detection, anal. and isolation of nascent **proteins**)
RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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(2) Kudlicki; J Mol Biol 1994, V244, P319 HCAPLUS
(3) Rothschild; US 5643722 A 1997 HCAPLUS
(4) Turcatti; J Biol Chem 1996, V271(33), P19991 HCAPLUS

L60 ANSWER 26 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2001:84778 HCAPLUS
DN 135:149273
TI Structure determination of **protein folds** using the **cell-free synthesis** and NMR spectroscopy
AU **Kigawa, Takanori; Yokoyama, Shigeyuki**
CS Genomic Sciences Center, RIKEN, Japan
SO Jikken Igaku (2000), 18(18), 2464-2468
CODEN: JIIGEF; ISSN: 0288-5514
PB Yodosha
DT Journal; General Review
LA Japanese
CC **9-0** (**Biochemical Methods**)
Section cross-reference(s): 6
AB A review with 11 refs. on automation and fast detn. of **protein** structure, **protein folding**, and physiol. **function**(s) using **cell-free protein synthesis** system to manuf. target **protein**, and NMR to study the structure of the target **protein**.
ST review **protein** structure detn automation
IT **Secondary structure**
Tertiary structure
(**protein**; structure detn. of **protein folds** using **cell-free synthesis** and NMR spectroscopy)
IT **Protein folding**
(structure detn. of **protein folds** using

cell-free synthesis and NMR spectroscopy)

L60 ANSWER 27 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2001:76271** HCAPLUS
DN **134:219310**
TI Automated search of natively **folded protein** fragments for high-throughput structure determination in structural genomics
AU Kuroda, Yutaka; Tani, Kazutoshi; Matsuo, Yo; **Yokoyama, Shigeyuki**
CS Protein Research Group, Genomic Sciences Center (GSC), The Institute of Physical and Chemical Research (RIKEN), Kanagawa, 230-0045, Japan
SO Protein Science (2000), 9(12), 2313-2321
CODEN: PRCIEI; ISSN: 0961-8368
PB Cambridge University Press
DT Journal
LA English
CC **9-16** (**Biochemical Methods**)
Section cross-reference(s): 6
AB Structural genomic projects envision almost routine **protein** structure detns., which are currently imaginable only for small **proteins** with mol. wts. below 25,000 Da. For larger **proteins**, structural insight can be obtained by breaking them into small segments of amino acid sequences that can **fold** into native structures, even when isolated from the rest of the **protein**. Such segments are autonomously **folding** units (AFU) and have sizes suitable for fast structural analyses. Here, we propose to expand an intuitive procedure often employed for identifying biol. important **domains** to an automatic method for detecting putative **folded protein** fragments. The procedure is based on the recognition that large **proteins** can be regarded as a combination of independent **domains** conserved among diverse organisms. We thus have developed a program that reorganizes the output of BLAST searches and detects regions with a large no. of similar sequences. To automate the detection process, it is reduced to a simple geometrical problem of recognizing rectangular shaped elevations in a graph that plots the no. of similar sequences at each residue of a query sequence. We used our program to quant. corroborate the premise that segments with conserved sequences correspond to **domains** that **fold** into native structures. We applied our program to a test data set composed of 99 amino acid sequences contg. 150 segments with structures listed in the **Protein** Data Bank, and thus known to **fold** into native structures. Overall, the fragments identified by our program have an almost 50% probability of forming a native structure, and comparable results are obsd. with sequences contg. **domain** linkers classified in SCOP. Furthermore, we verified that our program identifies AFU in libraries from various organisms, and we found a significant no. of AFU candidates for structural anal., covering an estd. 5 to 20% of the genomic databases. Altogether, these results argue that methods based on sequence similarity can be useful for dissecting large **proteins** into small autonomously **folding domains**, and such methods may provide an efficient support to structural genomics projects.
ST **protein** conformation **folding** sequence homol program
IT Computer program
(PASS; automated search of natively **folded protein** fragments for high-throughput structure detn. in structural genomics)
IT **Protein folding**
Protein sequences
(automated search of natively **folded protein** fragments for high-throughput structure detn. in structural genomics)
IT Peptides, analysis
Proteins, general, analysis
RL: ANT (Analyte); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study)
(automated search of natively **folded protein**

fragments for high-throughput structure detn. in structural genomics)
IT **Protein** sequences
(homol.; automated search of natively **folded protein**
fragments for high-throughput structure detn. in structural genomics)
IT **Conformation**
(**protein**; automated search of natively **folded**
protein fragments for high-throughput structure detn. in
structural genomics)
RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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(2) Altschul, S; Nucleic Acids Res 1997, V25, P3389 HCAPLUS
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(10) Corpet, F; Nucleic Acids Res 1998, V26, P323 HCAPLUS
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(32) Orengo, C; Nature 1994, V372, P631 HCAPLUS
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(35) Shapiro, L; Structure 1998, V6, P265 HCAPLUS
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(38) Wuthrich, K; Nat Struct Biol Suppl 1998, V5, P492 HCAPLUS
(39) Yokoyama, S; Nat Struct Biol 2000, V7(Suppl), P943
(40) Yu, H; Science 1992, V258, P1665 HCAPLUS

L60 ANSWER 28 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2000:900304** HCAPLUS
DN **134:27258**
TI Method and multi-channel membrane device for high throughput **cell free protein synthesis**
IN Metzler, Thomas; Schels, Hans
PA Roche Diagnostics G.m.b.H., Germany
SO Eur. Pat. Appl., 14 pp.
CODEN: EPXXDW
DT Patent
LA German
IC ICM C12M001-40

ICS C12P021-02; B01J019-24; B01J019-00
CC **9-1** (**Biochemical Methods**)
Section cross-reference(s): **3**, 6, 7, 16
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1061128	A1	20001220	EP 1999-111762	19990618
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	CA 2340712	AA	20001228	CA 2000-2340712	20000610
	WO 2000078444	A2	20001228	WO 2000-EP5378	20000610
	WO 2000078444	A3	20010426		
	W: CA, JP, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	EP 1126912	A1	20010829	EP 2000-935201	20000610
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2003502146	T2	20030121	JP 2001-504498	20000610
PRAI	EP 1999-111762	A	19990618		
	WO 2000-EP5378	W	20000610		

AB The invention concerns a method and a device for high throughput biochem. **synthesis** esp. **cell free protein synthesis** that contains an outer vessel for the **transcription/translation** reagent supply; and an inner part that is a microtiter plate or similar multi-channel holder with semipermeable membrane bottom and used for the **transcription/translation** reaction components. The inner part contains 2-1000 wells; the vol. of the wells is 50 .mu.l - 10 mL. **Protein**-binding compds., e.g. avidin, streptavidin are immobilized on the inner walls of the wells for the facile purifn. of the **synthesized** tag-contg. **proteins**. The wells are capped or covered with foil. The system is on a shaker and/or stirred with magnetic stirrers inside the wells. The supply of reagents is maintained via diffusion. Thus two model **proteins, green fluorescence protein** and chloramphenicol-acetyltransferase were **synthesized** using **E.coli** lysate and 40 wells of a microtiterplate for each **protein**. **Transcription/translation** reagents, plasmid , **E.coli** lysate, T7 RNA polymerase were applied into the wells; the same reagent supply was used for both **proteins**. After 20 h of incubation at 30.degree.C and shaking, the **proteins** were measured in ech well.

ST high throughput **cell free protein synthesis** multichannel membrane device

IT **Proteins**, specific or class
RL: BPN (Biosynthetic preparation); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(**green fluorescent**; method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT **Transcription**, genetic
Translation, genetic
(in vitro; method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT **Escherichia coli**
(lysate; method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT Apparatus
Diffusion
Immobilization, biochemical
Microtiter plates
Plasmid **vectors**
Shaking apparatus

(method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT **Fusion proteins (chimeric proteins)**
RL: BPN (Biosynthetic preparation); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT Avidins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT Membranes, nonbiological
(semipermeable; method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT **Proteins**, general, preparation
RL: PUR (Purification or recovery); PREP (Preparation)
(sepn.; method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT Mixers (processing apparatus)
(stirrers; method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT 9040-07-7P, Chloramphenicol-acetyltransferase
RL: BPN (Biosynthetic preparation); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT 9013-20-1, Streptavidin
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(method and multi-channel membrane device for high throughput **cell free protein synthesis**)

IT 9014-24-8
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(phage T7; method and multi-channel membrane device for high throughput **cell free protein synthesis**)

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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L60 ANSWER 29 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2000:895491** HCAPLUS
DN **135:222877**
TI Structural genomics projects in Japan
AU **Yokoyama, S.**; Matsuo, Y.; Hirota, H.; **Kigawa, T.**; Shirouzu, M.; Kuroda, Y.; Kurumizaka, H.; Kawaguchi, S.; Ito, Y.; Shibata, T.; Kainosho, M.; Nishimura, Y.; Inoue, Y.; Kuramitsu, S.
CS Tsurumi-ku, 1-7-22 Suehiro-cho, RIKEN Genomic Sciences Center, Yokohama, 230-0045, Japan
SO Progress in Biophysics & Molecular Biology (2000), 73(5), 363-376
CODEN: PBIMAC; ISSN: 0079-6107

PB Elsevier Science Ltd.
DT Journal; General Review
LA English
CC 6-0 (General Biochemistry)
AB A review with 22 refs. The "**Protein Folds** Project" and the "Structurome" project are described. Topics discussed include: **cell-free protein synthesis**; the large-scale NMR facility (NMR Farm); anal. of **protein function**; anal. of **protein-protein** interactions; selection of targets for structure detn.; identification of **protein domains**.
ST review structural genomics project Japan; **protein** structure **function** detn review
IT **Protein motifs**
(identification; structural genomics projects in Japan)
IT **Conformation**
(**protein**, detn. by NMR; structural genomics projects in Japan)
IT **Proteins**, general, biological studies
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(structure-**function** detn.; structural genomics projects in Japan)
RE.CNT 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Altschul, S; J Mol Biol 1990, V215, P403 HCAPLUS
(2) Bartel, P; Nat genetic 1996, V12, P72 HCAPLUS
(3) Brenner, S; Nucleic Acids Res 1999, V27, P251 HCAPLUS
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L60 ANSWER 30 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2000:891657 HCAPLUS
DN 134:4063
TI Large-scale preparation of **proteins** by the **cell-free synthesis**
AU **Kigawa, Takanori**
CS RIKEN, Japan
SO Seibutsu Butsuri (2000), 40(6), 391-394
CODEN: SEBUAL; ISSN: 0582-4052
PB Nippon Seibutsu Butsuri Gakkai
DT Journal; General Review
LA Japanese
CC 16-0 (Fermentation and Bioindustrial Chemistry)

AB A review with 11 refs. on improvement of **protein** prepn. systems contg. **Escherichia coli** exts. and method for regioselective introduction of non-natural amino acids.
ST review **protein cell free** manuf; Escherichia ext **protein** manuf review
IT **Escherichia coli**
Translation, genetic
(large-scale prepn. of **proteins** by the **cell-free synthesis**)
IT **Proteins**, general, preparation
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(large-scale prepn. of **proteins** by the **cell-free synthesis**)

L60 ANSWER 31 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2000:510868** HCAPLUS
DN **134:144124**
TI Large-scale purification of **recombinant green fluorescent protein** from **Escherichia coli**
AU Gonzalez, Daniel G.; Ward, William W.
CS Department of Biochemistry and Microbiology, Cook College, Rutgers University, New Brunswick, NS, 08901-8525, Can.
SO Methods in Enzymology (2000), 305(Bioluminescence and Chemiluminescence, Pt. C), 212-223
CODEN: MENZAU; ISSN: 0076-6879
PB Academic Press
DT Journal
LA English
CC **9-16** (**Biochemical Methods**)
AB **Green fluorescent protein** (**GFP**) has been shown to form without the need for any cofactors or specific posttranslational processing from the native jellyfish in a **cell-free translation** system. It has been demonstrated, however, that mol. oxygen is necessary for chromophore formation. Its fluorescence properties allow for easy visualization and quantitation of **GFP** in a nondestructive manner. As a result, **GFP** has been used increasingly as a **fusion** partner for use in monitoring **expression**. The fluorescence from **GFP** may also be used to assay for a valuable **recombinant protein** nondestructively. **GFP** can be employed as a model **protein** to develop methods for **protein** purifn. It can also be utilized to demonstrate **protein** purifn. concepts in an instructional setting. Protocols used are hereby described. (c) 2000 Academic Press.
ST purifn **recombinant green fluorescence protein Escherichia coli**
IT **Proteins**, specific or class
RL: PUR (Purification or recovery); PREP (Preparation)
(**green fluorescent**; large-scale purifn. of **recombinant green fluorescent protein** from **Escherichia coli**)
IT **Escherichia coli**
(large-scale purifn. of **recombinant green fluorescent protein** from **Escherichia coli**)
IT **Proteins**, general, preparation
RL: PUR (Purification or recovery); PREP (Preparation)
(large-scale purifn. of **recombinant green fluorescent protein** from **Escherichia coli**)
RE.CNT 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE
(1) Anon; personal communication from D Davis
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L60 ANSWER 32 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2000:336515 HCAPLUS
DN 133:146435
TI Effects of anticodon 2'-O-methylations on tRNA codon recognition in an **Escherichia coli cell-free** translation
AU Satoh, Akira; Takai, Kazuyuki; Ouchi, Ryosuke; **Yokoyama, Shigeyuki**; Takaku, Hiroshi
CS Department of Industrial Chemistry, Chiba Institute of Technology, Chiba, 275-0016, Japan
SO RNA (2000), 6(5), 680-686
CODEN: RNARFU; ISSN: 1355-8382
PB Cambridge University Press
DT Journal
LA English
CC 6-2 (General Biochemistry)
AB The methylation of 2'-hydroxyl groups is one of the most common posttranscriptional modifications of naturally occurring stable RNA mols. Some tRNA species have a 2'-O-Me nucleoside at the first position of the anticodon, and it was suggested that this modification stabilizes the codon-anticodon duplex. However, no tRNA species have been found to have the modification at the second or third position of the anticodon. In the present study, we measured the effects of anticodon 2'-O-methylation on the codon-reading efficiencies of the anticodon variants of the unmodified forms of **Escherichia coli** tRNA1Ser, using a **cell-free protein synthesis** assay. The modification of C in the first position of the anticodon into 2'-O-methylcytidine increased the efficiency of reading the G-ending codon. On the other hand, the modifications of the second and/or third positions were detrimental to the codon-reading activity. Thus, 2'-hydroxyl groups at the second and third positions of the anticodon may have some role in the translation reaction, and this may be the reason why 2'-O-Me nucleosides are not found in these positions within natural tRNA species.
ST anticodon methylation tRNA codon recognition
IT Codons
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL

(Biological study); PROC (Process)
(UCG and GAG recognition; **functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

IT Molecular recognition
Translation, genetic
tRNA sequences
(**functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

IT Anticodons
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(**functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

IT Structure-activity relationship
(of tRNA anticodons; **functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

IT RNA
(processing; **functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

IT tRNA
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)
(serine-specific UGA; **functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

IT 287121-69-1DP, derivs. contg. 2'-O-methylated and 2'-deoxy anticodon bases
287121-70-4DP, derivs. contg. 2'-O-methylated and 2'-deoxy anticodon bases
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)
(**functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

IT 65-46-3, Cytidine 951-77-9, 2'-Deoxycytidine 2140-72-9, 2'-O-Methylcytidine
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(of anticodon; **functional** role of anticodon 2'-O-methylation on tRNA codon recognition studied using anticodon-modified derivs. of **synthetic** unmodified **Escherichia coli** tRNASer)

RE.CNT 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L60 ANSWER 33 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **2000:263884** HCAPLUS
DN **133:306799**
TI Towards improved applications of **cell-free** **protein** biosynthesis - the influence of mRNA structure and suppressor tRNAs on the efficiency of the system
AU Gerrits, Michael; Merk, Helmut; Stiege, Wolfgang; Erdmann, Volker A.
CS Institut fur Biochemie, Freie Universitat Berlin, Berlin, D-14195, Germany
SO NATO Science Series, 3: High Technology (1999), 70(RNA Biochemistry and Biotechnology), 335-345
CODEN: NSSTFF; ISSN: 1388-6576
PB Kluwer Academic Publishers
DT Journal
LA English
CC 6-1 (General Biochemistry)
Section cross-reference(s): 7, **9**
AB The **cell-free protein** biosynthesis has the potential to become a powerful technol. for the biochem. research in particular in the detn. of the structure and **function** of **proteins**. The no. of possible applications is rising with the obtainable yields and with the expanded feasibility of introducing modified amino acids into **proteins**. Here we describe the influence of two RNA **translation** components, the mRNA and the suppressor tRNA, on the efficiency of **protein** biosynthesis. It is shown that the rate limiting factor of the **cell-free** **translation** of the two **proteins** dihydrofolate reductase (DHFR) and fatty acid binding **protein** (FABP) is not the initiation or termination step. The efficiency of peptide bond formation in the nascent **protein** varies between the two genes but is independent on the size of the coding sequences. The poor **translation** of DHFR can be improved when its coding sequence is **fused** with a part of the more efficiently **translated** FABP gene. We compared different amber suppressor tRNAs on the level of **translational** efficiency and aminoacylation capacity. Our results show that in most cases the aminoacylation rate of the tRNAs is not the limiting factor of suppression. An **E. coli** tRNALeuCUA exhibits the highest **translational** efficiency of the examd.

tRNAs. So this tRNALeuCUA may be a starting point to construct more efficient tRNAs for the introduction of unnatural amino acids into **proteins** in the in vitro **translation** system by eliminating the **synthetase** mediated aminoacylation of the tRNA.

ST **protein translation synthesis** mRNA structure tRNA suppressor; dihydrofolate reductase **translation synthesis** mRNA structure tRNA suppressor; FABP **protein translation synthesis** mRNA structure tRNA suppressor

IT **Proteins**, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); SPN (Synthetic preparation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation); PROC (Process)
(FABP (fatty acid-binding **protein**); towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

IT tRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(amber suppressor; towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

IT tRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(isoleucine-specific GAU; towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

IT tRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(suppressor; towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

IT Aminoacylation
Translation, genetic
(towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

IT **Proteins**, general, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); SPN (Synthetic preparation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation); PROC (Process)
(towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

IT mRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

IT 9002-03-3P, Dihydrofolate reductase
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); SPN (Synthetic preparation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation); PROC (Process)
(towards improved applications of **cell-free protein** biosynthesis - influence of mRNA structure and suppressor tRNAs on efficiency of system)

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L60 ANSWER 34 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 2000:183931 HCAPLUS
DN 132:319466
TI An easy **cell-free protein synthesis** system dependent on the addition of crude **Escherichia coli** tRNA
AU Kanda, Takayuki; Takai, Kazuyuki; **Yokoyama, Shigeyuki**; Takaku, Hiroshi
CS Department of Industrial Chemistry, Chiba Institute of Technology, Chiba, 275-0016, Japan
SO Journal of Biochemistry (Tokyo) (2000), 127(1), 37-41
CODEN: JOBIAO; ISSN: 0021-924X
PB Japanese Biochemical Society
DT Journal
LA English
CC **9-16** (**Biochemical Methods**)
AB The **protein-synthesizing** S30 ext. of **Escherichia coli** contains tRNA, which limits its applications in **cell-free protein synthesis**. Here, we show that at least Arg- and Ser-acceptor activities can be removed from a std. S30 ext. by treatment with an immobilized RNase A resin. This RNase-treated ext. exhibits no **protein synthesis** activity, but regains it when supplied with crude **E. coli** tRNA and a small amt. of human placental RNase inhibitor. The **protein synthesis** is dependent on the addn. of tRNA in the presence of the RNase inhibitor. Chloramphenicol acetyltransferase was **synthesized** with this system and found to be active.
ST **protein synthesis** system Escherichia tRNA
IT **Escherichia coli**
Translation, genetic
(easy **cell-free protein synthesis** system dependent on addn. of crude **Escherichia coli** tRNA)
IT tRNA
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(easy **cell-free protein synthesis** system dependent on addn. of crude **Escherichia coli**

tRNA)
IT 9040-07-7, Chloramphenicol acetyltransferase
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(easy **cell-free protein synthesis**
system dependent on addn. of crude **Escherichia coli**
tRNA)
RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L60 ANSWER 35 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:156625 HCAPLUS
DN 130:219543
TI Large-scale **protein** preparation using the **cell-free synthesis**
AU **Kigawa, Takanori**; Yabuki, Takashi; **Yokoyama, Shigeyuki**
CS Cell. Signaling Lab., Inst. Phys. Chem. Res., Wako, 351-0198, Japan
SO Tanpakushitsu Kakusan Koso (1999), 44(4), 598-605
CODEN: TAKKAJ; ISSN: 0039-9450
PB Kyoritsu Shuppan
DT Journal; General Review
LA Japanese
CC 6-0 (General Biochemistry)
AB A review with 21 refs., on method for **cell-free synthesis** of **proteins** and its application to prepn. of amino acid-selective- and site-specific- labeled **proteins**, and development and application of large-scale **synthesis** system using dialysis. Method for tyrosine-selective labeling of Ras **protein** by **cell-free synthesis** is also discussed.
ST review **protein** prepn **cell free** system; isotope labeling **protein synthesis** review
IT **Proteins**, specific or class

RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(labeled; large-scale **protein** prepn. using **cell free synthesis**)

IT Genetic engineering
Translation, genetic
(large-scale **protein** prepn. using **cell free synthesis**)

IT **Proteins**, general, preparation
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(large-scale **protein** prepn. using **cell free synthesis**)

L60 ANSWER 36 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:17197 HCAPLUS
DN 130:249005
TI **Cell-free** production and stable-isotope labeling of milligram quantities of **proteins**
AU **Kigawa, Takanori**; Yabuki, Takashi; Yoshida, Yasuhiko; Tsutsui, Michio; Ito, Yutaka; Shibata, Takehiko; **Yokoyama, Shigeyuki**
CS Cellular Signaling Laboratory, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama, 351-0198, Japan
SO FEBS Letters (1999), 442(1), 15-19
CODEN: FEBLAL; ISSN: 0014-5793
PB Elsevier Science B.V.
DT Journal
LA English
CC **9-8 (Biochemical Methods)**
AB We have improved the productivity of an **Escherichia coli** **cell-free protein synthesis** system. First, creatine phosphate and creatine kinase were used as the energy source regeneration system, and the other components of the reaction mixt. were optimized. Second, the **E. coli** S30 **cell** ext. was condensed by dialysis against a polyethylene glycol soln. to increase the rate of **synthesis**. Third, during the **protein synthesis**, the reaction mixt. was dialyzed against a low-mol.-wt. substrate soln. to prolong the reaction. Thus, the yield of chloramphenicol acetyltransferase was raised to 6 mg/mL of reaction mixt. Stable-isotope labeling of a **protein** with 13C/15N-labeled amino acids for NMR spectroscopy was achieved by this method.
ST **cell free** prodn isotope labeling **protein**

IT Dialysis
Escherichia coli
NMR (nuclear magnetic resonance)
(**cell-free** prodn. and stable-isotope labeling of milligram quantities of **proteins**)

IT **Proteins**, general, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**cell-free** prodn. and stable-isotope labeling of milligram quantities of **proteins**)

IT Ras **proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**cell-free** prodn. and stable-isotope labeling of milligram quantities of **proteins**)

IT Isotopes
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**cell-free** prodn. and stable-isotope labeling of milligram quantities of **proteins**)

IT 9040-07-7, Chloramphenicol acetyltransferase

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**cell-free** prodn. and stable-isotope labeling of milligram quantities of **proteins**)
IT 67-07-2, Creatine phosphate 9001-15-4, Creatine kinase
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**cell-free** prodn. and stable-isotope labeling of milligram quantities of **proteins**)
RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L60 ANSWER 37 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1998:780895 HCAPLUS
DN 130:178868
TI Knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides
AU Kanda, Takayuki; Takai, Kazuyuki; **Yokoyama, Shigeyuki**; Takaku, Hiroshi
CS Faculty of Engineering, Department of Industrial Chemistry, Chiba Institute of Technology, Narashino, Chiba, 275-0016, Japan
SO FEBS Letters (1998), 440(3), 273-276
CODEN: FEBLAL; ISSN: 0014-5793
PB Elsevier Science B.V.
DT Journal
LA English
CC 6-1 (General Biochemistry)
AB Methods for the prepn. of an **Escherichia coli** tRNA mixt. lacking one or a few specific tRNA species can be the basis for future applications of **cell-free protein synthesis**. We demonstrate here that virtually a single tRNA species in a crude **E. coli** tRNA mixt. can be knocked out by an antisense (complementary) oligodeoxyribonucleotide. One out of five oligomers complementary to tRNAAsp blocked the aspartylation almost completely, while minimally affecting the aminoacylation with other 13 amino acids tested. This 'knockout' tRNA behaved similarly to the untreated tRNA in a **cell-free** translation of an mRNA lacking Asp codons.
ST tRNA antisense oligodeoxyribonucleotide translation aminoacylation RNase H

IT tRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(aspartic acid-specific; knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides)
IT Aminoacylation
Translation, genetic
(knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides)
IT DNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides)
IT tRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides)
IT Antisense oligonucleotides
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides)
IT 9050-76-4, Ribonuclease H
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides)
IT 220419-85-2 220419-98-7 220420-00-8 220420-01-9 220420-03-1
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(knocking out a specific tRNA species within unfractionated **Escherichia coli** tRNA by using antisense (complementary) oligodeoxyribonucleotides)
RE.CNT 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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L60 ANSWER 38 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1998:590136 HCAPLUS
DN 129:299303
TI A **cell-free protein synthesis** system lacking specific tRNA species
AU Kanda, Takayuki; Takai, Kazuyuki; **Yokoyama, Shigeyuki**; Takaku, Hiroshi
CS Department of Industrial Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba, 275-0016, Japan
SO Nucleic Acids Symposium Series (1998), 39, 167-168
CODEN: NACSD8; ISSN: 0261-3166
PB Oxford University Press
DT Journal
LA English
CC 6-1 (General Biochemistry)
AB Prepn. of a **protein** with an unnatural or a labeled amino acid residue was achieved by **cell-free protein synthesis** supplied with an artificially-prepd. aminoacyl-tRNA that suppresses a stop codon. Sense codons cannot be used for this purpose, because a competing tRNA can also insert an unwanted natural amino acid at the position of the intended artificial amino acid. To utilize sense codons for the artificial amino acids, we have developed a strategy for the prepn. of **Escherichia coli** tRNA mixts. lacking the activity of a single tRNA species (tRNAAsp or tRNAPhe) based on oligonucleotide hybridization. In the present study, we demonstrated that these "knockout" tRNA mixts. cannot decode a codon corresponding to the inactivated tRNA, but can decode almost all of the other codons.
ST translation artificial amino acid tRNA
IT Codons
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(GAC and UUC; **cell-free protein synthesis** system lacking specific tRNA species for utilizing sense codons for the artificial amino acids)
IT **Translation, genetic**
(**cell-free protein synthesis** system lacking specific tRNA species for utilizing sense codons for the artificial amino acids)
IT tRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(**cell-free protein synthesis** system lacking specific tRNA species for utilizing sense codons for the artificial amino acids)
IT 56-87-1P, L-Lysine, preparation 60-18-4P, L-Tyrosine, preparation 72-19-5P, L-Threonine, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(**cell-free protein synthesis** system lacking specific tRNA species for utilizing sense codons for the artificial amino acids)
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L60 ANSWER 39 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN

AN 1998:477959 HCAPLUS
DN 129:200095
TI Dual amino acid-selective and site-directed stable-isotope labeling of the human c-Ha-Ras **protein** by **cell-free synthesis**
AU Yabuki, Takashi; **Kigawa, Takanori**; Dohmae, Naoshi; Takio, Koji; Terada, Tohru; Ito, Yutaka; Laue, Ernest D.; Cooper, Jonathan A.; Kainosho, Masatsune; **Yokoyama, Shigeyuki**
CS Cellular Signaling Laboratory, The Institute of Physical and Chemical Research (RIKEN), Saitama, 351-01, Japan
SO Journal of Biomolecular NMR (1998), 11(3), 295-306
CODEN: JBNME9; ISSN: 0925-2738
PB Kluwer Academic Publishers
DT Journal
LA English
CC **9-12** (**Biochemical Methods**)
Section cross-reference(s): 3, 7
AB We developed two methods for stable-isotope labeling of **proteins** by **cell-free synthesis**. Firstly, we applied **cell-free synthesis** to the dual amino acid-selective 13C-15N labeling method, originally developed for in vivo systems by Kainosho and co-workers. For this purpose, we took one of the advantages of a **cell-free protein synthesis** system: the amino acid-selective stable-isotope labeling is **free** of the isotope scrambling problem. The targets of selective observation were Thr35 and Ser39 in the "effector region" (residues 32-40) of the Ras **protein** complexed with the Ras-binding **domain** of c-Raf-1 (Raf RBD) (the total mol. mass is about 30 kDa). Using a 15-mL **Escherichia coli cell-free** system, which was optimized to produce about 0.4 mg of Ras **protein** per 1-mL reaction, with 2 mg each of DL-[13C']proline and L-[15N]threonine, we obtained about 6 mg of Ras **protein**. As the Pro-Thr sequence is unique in the Ras **protein**, the Thr35 cross peak of the Ras.cntdot.Raf RBD complex was unambiguously identified by the 2D 1H-15N HNCO expt. The Ser39 cross peak was similarly identified with the [13C']Asp/[15N]Ser-selectively labeled Ras **protein**. There were no isotope scrambling problems in this study. Secondly, we have established a method for producing a milligram quantity of site-specifically stable-isotope labeled **protein** by a **cell-free** system involving amber suppression. The **E. coli** amber suppressor tRNATyrCUA (25 mg) was prepd. by in vitro transcription with T7 RNA polymerase. We aminoacylated the tRNATyrCUA transcript with purified **E. coli** tyrosyl-tRNA **synthetase**, using 2 mg of L-[15N]tyrosine. In the gene encoding the Ras **protein**, the codon for Tyr32 was changed to an amber codon (TAG). This template DNA and the [15N]Tyr-tRNATyrCUA were reacted for 30 min in 30 mL of **E. coli cell-free** system. The subsequent purifn. yielded 2.2 mg of [15N]Tyr32-Ras **protein**. In the 1H-15N HSQC spectrum of the labeled Ras **protein**, only one cross peak was obsd., which was unambiguously assigned to Tyr32.
ST isotope labeling cHa Ras **protein**; **cell free synthesis** Ras **protein** Raf; HSQC NMR Ras labeled Raf RBD
IT Termination factors (**protein** formation)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(RF-1 (release factor 1); dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT Promoter (genetic element)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(T7; dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)

IT Aminoacylation
Enzyme functional sites
Escherichia coli
Gel electrophoresis
Synthesis
Translation, genetic
(dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT Isotopes
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT Ras **proteins**
RL: PRP (Properties); SPN (Synthetic preparation); PREP (Preparation)
(dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT Transcription, genetic
(in vitro; dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT NMR spectroscopy
(multinuclear, HSQC spectrum; dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT Plasmids
(pK7-RasY32am and pK7-Ras; dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT Laser ionization mass spectrometry
(photodesorption, matrix-assisted; dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT Laser desorption mass spectrometry
(photoionization, matrix-assisted; dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT tRNA
RL: BPN (Biosynthetic preparation); PRP (Properties); RCT (Reactant); BIOL (Biological study); PREP (Preparation); RACT (Reactant or reagent)
(tyrosine-specific, **E.coli** amber suppressor tRNAtyrCUA; dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT 9014-24-8D, RNA polymerase, derivs.
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT 9023-45-4P, **Synthetase**, tyrosyl-transfer ribonucleate
RL: CAT (Catalyst use); PUR (Purification or recovery); PREP (Preparation); USES (Uses)
(dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free synthesis**)
IT 59935-32-9, L-Serine-15N 80681-09-0, L-Threonine-15N 81201-97-0, L-Aspartic-1-13C acid 212258-93-0, Proline-carboxy-13C
RL: PRP (Properties); RCT (Reactant); RACT (Reactant or reagent)
(dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free**

synthesis)
IT 139691-76-2P
RL: PRP (Properties); SPN (Synthetic preparation); PREP (Preparation)
(dual amino acid-selective and site-directed stable-isotope labeling of human c-Ha-Ras **protein** by **cell-free** **synthesis**)
RE.CNT 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L60 ANSWER 40 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1998:295237 HCAPLUS
DN 129:50985
TI Removing tRNA from a **cell-free protein** **synthesis** system for use in **protein** production
AU Kanada, Takayuki; Takai, Kazuyuki; **Yokoyama, Shigeyuki**; Takaku, Hiroshi
CS Dep. of Industrial Chemistry, Chiba Institute of Technology, Narashino, 275, Japan
SO Nucleic Acids Symposium Series (1997), 37(Symposium on Nucleic Acids Chemistry, 1997), 319-320

CODEN: NACSD8; ISSN: 0261-3166
PB Oxford University Press
DT Journal
LA English
CC 6-2 (General Biochemistry)
AB The **cell-free** system for biosynthesis of **proteins** is becoming an important tool for **protein** engineering. In particular, introduction of the unnatural amino acids is achieved though **cell-free protein synthesis** with the use of chem. acylated tRNA that recognizes a specific codon. In the original method, however, it was difficult to control the system through changing tRNA compn., as the endogenous tRNAs are involved in the reaction. Thus, in the present study, we **digested** the tRNA within **Escherichia coli** S30 ext. with resin-bound RNase A, and estd. the **protein synthesis** activity. It was revealed that this **digestion** process does not damage the activity, if a protease inhibitor, phenylmethylsulfonyl fluoride (PMSF), is present in the **digestion** reaction.
ST tRNA translation **protein** engineering RNase A
IT **Protein** engineering
Translation, genetic
(removing tRNA from a **cell-free protein synthesis** system for use in **protein** prodn.)
IT tRNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(removing tRNA from a **cell-free protein synthesis** system for use in **protein** prodn.)
IT 9001-99-4, Ribonuclease
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(A; removing tRNA from a **cell-free protein synthesis** system for use in **protein** prodn.)
IT 329-98-6, Phenylmethylsulfonyl fluoride
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(removing tRNA from a **cell-free protein synthesis** system for use in **protein** prodn.)
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Kanda, T; Nucleic Acids Res Sym Ser 1996, V35, P179
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L60 ANSWER 41 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **1997:356920** HCAPLUS
DN **127:106257**
TI Correspondence: **Cell-free expression** of a **GFP fusion protein** allows quantitation in vitro and in vivo
AU Kahn, Theodore W.; Beachy, Roger N.; Falk, Matthias M.
CS Department Cell Biology, Scripps Research Institute, La Jolla, CA, 92037, USA
SO Current Biology (1997), 7(4), R207-R208
CODEN: CUBLE2; ISSN: 0960-9822
PB Current Biology
DT Journal
LA English
CC **9-16 (Biochemical Methods)**
AB When a **GFP (green fluorescent**

protein) **fusion protein** is translated in vitro under std. conditions, the **GFP** portion **folds** efficiently and becomes fluorescent. This provides a convenient method for monitoring in vitro translation efficiency of a **fusion protein**, and to screen for improving mutants of **GFP**.

ST cell **GFP fusion protein**

IT **Proteins**, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(**green fluorescent, fusion protein**; correspondence: **cell-free expression** of a **GFP fusion protein** allows quantitation in vitro and in vivo)

L60 ANSWER 42 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN **1997:228751** HCAPLUS
DN **126:313776**
TI **Protein** engineering of de novo **protein** with predesigned structure and activity
AU Dolgikh, Dmitry A.; Gabrielian, Andrei E.; Uversky, Vladimir N.; Kirpichnikov, Michael P.
CS Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 117984, Russia
SO Applied Biochemistry and Biotechnology (1997), Volume Date 1996, 61(1/2, Biocatalysis-95), 85-96
CODEN: ABIBDL; ISSN: 0273-2289
PB Humana
DT Journal
LA English
CC 6-3 (General Biochemistry)
Section cross-reference(s): **3**
AB The de novo **protein** albebetin has been engineered (J. Mol. Biol. 1992, 225, 927-931) to form a predesigned tertiary **fold** that has not yet been obsd. in natural **proteins**. Anal. of albebetin **expressed** in a **cell-free** system and in **Escherichia coli** revealed its compactness, relative stability, and the secondary structure close to the predesigned one. The blast-transforming biol. activity of human interferon was grafted to albebetin by attachment of an eight amino acid interferon fragment to the N-terminus of albebetin next to its first methionine residue. The chimeric **protein** was **expressed** in a wheat germ **cell-free translation** system and tested for its structural properties, receptor binding, and biol. activity. According to the tests, albebetin incorporating the active interferon fragment has a compact and relatively stable structure, and binds the murine thymocyte receptor effectively. It activates the blast transformation reaction of thymocyte **cells** even more efficiently than human interferon at low concns.

ST albebetin interferon conjugate **recombinant** receptor binding

IT **Proteins**, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(albebetin, **fusion protein** with interferon .alpha.2 fragment; **protein** engineering of de novo **protein** with predesigned structure and activity)

IT **Escherichia coli**
(albebetin-interferon conjugate prodn. with; **protein** engineering of de novo **protein** with predesigned structure and activity)

IT **DNA sequences**
(for interferon .alpha.2-albebetin **fusion protein**)

IT **Protein** sequences
(of interferon .alpha.2-albebetin **fusion protein**)
IT Interferons
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(.alpha.2, **fusion protein** with albebetin; **protein** engineering of de novo **protein** with predesigned structure and activity)
IT 148522-27-4P
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(amino acid sequence; **protein** engineering of de novo **protein** with predesigned structure and activity)
IT 189355-70-2
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; **protein** engineering of de novo **protein** with predesigned structure and activity)

L60 ANSWER 43 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1996:554280 HCAPLUS
DN 125:188549
TI Codon-reading specificity of an unmodified form of **Escherichia coli** tRNA1Ser in **cell-free protein synthesis**
AU Takai, Kazuyuki; Takaku, Hiroshi; **Yokoyama, Shigeyuki**
CS Dep. Biophys. Biochem., Univ. Tokyo, Tokyo, 113, Japan
SO Nucleic Acids Research (1996), 24(15), 2894-2899
CODEN: NARHAD; ISSN: 0305-1048
PB Oxford University Press
DT Journal
LA English
CC 6-1 (General Biochemistry)
Section cross-reference(s): 3
AB Unmodified tRNA mols. are useful for many purposes in **cell-free protein** biosynthesis, but there is little information about how the lack of tRNA posttranscriptional modifications affects the coding specificity for synonymous codons. In the present study, we prepd. an unmodified form of **Escherichia coli** tRNA1Ser, which originally has the cmo5UGA anticodon (cmo5U = uridine 5-oxyacetic acid) and recognizes the UCU, UCA and UCG codons. The codon specificity of the unmodified tRNA was tested in a **cell-free protein synthesis** directed by designed mRNAs under competition conditions with the parent tRNA1Ser. It was found that the unmodified tRNA with the UGA anticodon recognizes the UCA codon nearly as efficiently as the modified tRNA. The unmodified tRNA recognized the UCU codon with low, but detectable efficiency, whereas no recognition of the UCC and UCG codons was detected. Therefore, the absence of modifications makes this tRNA more specific to the UCA codon by remarkably reducing the efficiencies of wobble reading of other synonymous codons, without a significant decrease in the UCA reading efficiency.
ST codon specificity Escherichia tRNA1Ser **protein synthesis**
IT **Escherichia coli**
Translation, genetic
(codon-reading specificity of an unmodified form of **Escherichia coli** tRNA1Ser in **cell-free protein synthesis**)
IT Codon
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(codon-reading specificity of an unmodified form of **Escherichia**

coli tRNA1Ser in **cell-free protein synthesis**)

IT Ribonucleic acids, transfer
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(serine-specific 1, codon-reading specificity of an unmodified form of **Escherichia coli** tRNA1Ser in **cell-free protein synthesis**)

L60 ANSWER 44 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1996:512916 HCAPLUS
DN 125:162430
TI A highly efficient **cell-free protein synthesis** system from **Escherichia coli**
AU Kim, Dong-Myung; **Kigawa, Takanori**; Choi, Cha-Yong; **Yokoyama, Shigeyuki**
CS Interdisciplinary Program Biochemical Eng. and Technology, Seoul Natl. Univ., S. Korea
SO European Journal of Biochemistry (1996), 239(3), 881-886
CODEN: EJBCAI; ISSN: 0014-2956
PB Springer
DT Journal
LA English
CC **9-2** (**Biochemical Methods**)
AB The authors modified a **cell-free** coupled transcription/translation system from **Escherichia coli** with the T7 phage RNA polymerase, and achieved a productivity as high as 0.4 mg **protein**/mL reaction mixt. First, the authors found that the optimal concns. of phosphoenolpyruvate and poly(ethylene glycol) are interdependent; higher concns. of the former should be used at higher concns. of the latter. Second, the use of a condensed 30,000 g **cell** ext., in place of the conventional one, significantly increased the initial rate of **protein synthesis**. This phenomenon was demonstrated to be due to a reason other than elimination of inhibitory mol(s). from the ext. For this system with the condensed ext., the phosphoenolpyruvate and poly(ethylene glycol) concns. were again co-optimized, resulting in prodn. of chloramphenicol acetyltransferase at a productivity of 0.3 mg/mL. Finally, the productivity was further increased up to 0.4 mg/mL, by supplementation of the pool of amino acids. This improved **cell-free protein synthesis** system is superior in productivity to any other **cell-free** systems reported so far, including the continuous-flow **cell-free** system.
ST **cell free** translation system Escherichia
IT **Escherichia coli**
Translation, genetic
(highly efficient **cell-free protein synthesis** system from **Escherichia coli**)
IT Virus, bacterial
(T7, highly efficient **cell-free protein synthesis** system from **Escherichia coli**)
IT 9040-07-7P, Chloramphenicol acetyltransferase
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(highly efficient **cell-free protein synthesis** system from **Escherichia coli**)
IT 138-08-9, Phosphoenolpyruvic acid 9014-24-8, RNA polymerase 25322-68-3
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(highly efficient **cell-free protein synthesis** system from **Escherichia coli**)

L60 ANSWER 45 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN

AN 1995:871566 HCAPLUS
DN 123:250460
TI **Cell-free synthesis** and amino acid-selective stable isotope labeling of **proteins** for NMR analysis
AU **Kigawa, Takanori**; Muto, Yutaka; **Yokoyama, Shigeyuki**
CS Dep. Biophys. Biochem. Sch. Sci., Univ. Tokyo, Tokyo, 113, Japan
SO Journal of Biomolecular NMR (1995), 6(2), 129-34
CODEN: JBNME9; ISSN: 0925-2738
PB ESCOM
DT Journal
LA English
CC **9-5** (**Biochemical Methods**)
Section cross-reference(s): 6, 77
AB For the application of multidimensional NMR spectroscopy to larger **proteins**, it would be useful to perform selective labeling of one of the 20 amino acids. For some amino acids, however, amino acid metab. drastically reduces the efficiency and selectivity of labeling in in vivo **expression** systems. In the present study, a **cell-free protein synthesis** system was optimized, so that highly efficient and selective stable isotope labeling of **proteins** can be achieved in the absence of amino acid metab. The productivity of the **E. coli cell-free** coupled transcription-translation system was first improved, by about 5-**fold**, by using the T7 RNA polymerase for transcription and also by improving the translation conditions. Thus, about 0.1 mg **protein** per 1 mL reaction mixt. was **synthesized**. Then, this improved **cell-free** system was used for Asp- or Ser-selective 15N-labeling of the human c-Ha-Ras **protein**. With a 15 mL **cell-free** reaction, using <1 mg of 15N-labeled amino acid, 1 mg of the Ras **protein** was obtained. 1H-15N HSQC expts. confirmed that the Ras **protein** was efficiently labeled with high selectivity. These results indicate that this **cell-free protein synthesis** system is useful for NMR studies.
ST **protein** stable isotope labeling NMR analysis; **cell free protein synthesis** isotope labeling; nitrogen 15 labeling cHaRas **protein**
IT **Escherichia coli**
Nuclear magnetic resonance spectrometry
(**cell-free synthesis** and amino acid-selective stable isotope labeling of **proteins** for NMR anal.)
IT **Proteins**, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(**cell-free synthesis** and amino acid-selective stable isotope labeling of **proteins** for NMR anal.)
IT G **proteins** (guanine nucleotide-binding **proteins**)
RL: RCT (Reactant); RACT (Reactant or reagent)
(p21c-Ha-ras, **cell-free synthesis** and amino acid-selective stable isotope labeling of **proteins** for NMR anal.)
IT Isotope indicators
(stable, **cell-free synthesis** and amino acid-selective stable isotope labeling of **proteins** for NMR anal.)
IT 14390-96-6, Nitrogen 15, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(**cell-free synthesis** and amino acid-selective stable isotope labeling of **proteins** for NMR anal.)

L60 ANSWER 46 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN

AN 1994:4060 HCAPLUS
DN 120:4060
TI Labeling of **proteins** in **cell-free protein synthesis** system containing non-natural amino acid substrates
IN **Yokoyama, Shigeyuki**; **Kikawa, Takanori**; Tejima, Munehiro
PA Nippon Oxygen Co Ltd, Japan
SO Jpn. Kokai Tokkyo Koho, 9 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
IC ICM C12P021-00
ICS C12P021-00
ICA C12N015-09
CC **9-14** (**Biochemical Methods**)
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 05236987	A2	19930917	JP 1991-208321	19910820
JP 3317983	B2	20020826		
PRAI JP 1991-208321		19910820		

AB A method for labeling **proteins** with non-natural amino acid substrates in a **cell-free protein synthesis** system and a kit for the method are provided. The system employes codon-specific isoaccepting tRNA and the corresponding non-natural amino acids so that the labeling can be directed to specific triplet codon(s). The non-natural amino acids can be the natural amino acids having atoms substituted with a stable isotope or a radioisotope, enantiomeric side chains, or substituted side chains that cause changes of properties (e.g. mol. size, reactivity, hydrophobicity, fluorescence, hydrogen bonding, etc). A kit for **cell-free protein synthesis** contg. ribosomes, tRNAs, **enzymes**, isoaccepting tRNA bound with (non-)natural amino acids, and ribonucleotides and the **proteins** prepd. with the method are claimed. Prepn. of chlroamphenicol acetyl transferase (CAT) using **Escherichia coli** S30 ext. was shown, in which natural arginyl tRNA1, tRNA2, and tRNA3 and 15N-labeled tRNA4 were used. The resultant CAT contained 15N-Arg at position 213, but not the rest of Arg (at positions 18, 65, 74, and 124).

ST **protein** labeling artificial amino acid; tRNA isoaccepting **protein** labeling

IT Isotopes
Radioelements, uses
RL: ANST (Analytical study)
(amino acids labeled with, for **protein** labeling, isoaccepting tRNA in **cell-free protein synthesis** system in relation to)

IT **Translation, genetic**
(**cell-free**, isoaccepting tRNA in, for **protein** labeling)

IT Ribonucleic acids, transfer
RL: ANST (Analytical study)
(isoaccepting, in codon-specific labeling of **proteins**)

IT **Proteins**, biological studies
RL: BIOL (Biological study)
(labeling of, codon-specific, isoaccepting tRNA in)

IT Amino acids, uses
RL: USES (Uses)
(**proteins** labeling with, isoaccepting tRNA in)

IT Isomerism and Isomers
(optical, amino acids labeled with, for **protein** labeling, isoaccepting tRNA in **cell-free protein**

synthesis system in relation to)

L60 ANSWER 47 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1994:4034 HCAPLUS
DN 120:4034
TI Labeling of **proteins** with stable radioisotopes and kits for labeling
IN **Yokoyama, Shigeyuki**; **Kikawa, Takanori**; Tejima, Munehiro
PA Nippon Oxygen Co Ltd, Japan
SO Jpn. Kokai Tokkyo Koho, 6 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
IC ICM C12P021-00
ICA C12N015-09
CC **9-8** (**Biochemical Methods**)
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 05236986	A2	19930917	JP 1991-208322	19910820
JP 3145431	B2	20010312		
PRAI JP 1991-208322		19910820		

AB A method for labeling **proteins** with stable radioisotopes in a **cell-free**, **protein synthetic** system is described. The system is continuously supplied with amino acid substrates labeled with radioisotopes such as 13C and 15N and the product can be almost 100% labeled. It also contains the amino acid **synthesis** system, an inhibitor for amino acid metab., and ribonucleotides. Other **enzymes** from the **cell** origins are removed by, e.g., monoclonal antibodies. The method was demonstrated by **synthesis** of 15N-labeled CAT using the **Escherichia coli** S-30 ext.
ST **cell free protein synthesis** radioisotope labeling
IT **Proteins**, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(labeling of, with radioisotopes, **cell-free** **protein synthetic** system for)
IT Radioelements, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(**protein** labeling with, **cell-free** **protein synthetic** system for)

L60 ANSWER 48 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1992:610746 HCAPLUS
DN 117:210746
TI **Cell-free**, continuous **synthesis** of polypeptide
IN **Yokoyama, Shigeyuki**; Endo, Yaeta; **Kikawa, Takanori**
PA Japan
SO Jpn. Kokai Tokkyo Koho, 7 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
IC ICM C12P021-00
CC 16-4 (Fermentation and Bioindustrial Chemistry)
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 04200390	A2	19920721	JP 1990-334103	19901130
PRAI JP 1990-334103		19901130		

AB A **cell-free**, ribosome-contg., continuous system for

synthesis of polypeptide (excluding chem. **synthesis**) from mRNA is disclosed. In this system, the reaction substrates, e.g., ATP, GTP, amino acids, etc., are continuously supplied to the reaction chamber having minimal air content and the reaction mixt. is pumped through an ultrafiltration app. to sep. polypeptide products from the low-mol. substrates. The process minimizes foaming and therefore **protein** denaturation as compared to that of prior art. **Synthesis** of chloramphenicol acetyltransferase (CAT) was performed continuously for 17 h with a good yield.

ST **cell free** continuous polypeptide **synthesis**; foaming **free protein synthesis cell free**

IT Foaming
(**cell-free** and continuous **protein synthesis** system low in)

IT **Translation, genetic**
(**cell-free** and continuous system for, low-foaming)

IT Air
(min., in **cell-free** and continuous system for **protein synthesis, protein** denaturation minimization in relation to)

IT Denaturation
(**protein**, foaming in, **cell-free** and continuous system for **protein synthesis** avoiding, min. air content in relation to)

IT Peptides, preparation
Proteins, preparation
RL: RCT (Reactant); RACT (Reactant or reagent)
(**synthesis** of, **cell-free** and continuous system low in foaming for)

IT 9040-07-7
RL: RCT (Reactant); RACT (Reactant or reagent)
(**synthesis** of, **cell-free** and continuous system low in foaming for)

L60 ANSWER 49 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1991:581406 HCAPLUS
DN 115:181406
TI A continuous **cell-free protein synthesis** system for coupled transcription-translation
AU **Kigawa, Takanori; Yokoyama, Shigeyuki**
CS Fac. Sci., Univ. Tokyo, Tokyo, 113, Japan
SO Journal of Biochemistry (Tokyo, Japan) (1991), 110(2), 166-8
CODEN: JOBIAO; ISSN: 0021-924X
DT Journal
LA English
CC 16-1 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): **9**

AB A continuous **cell-free protein synthesis** system (Spirin, A. S. et al., 1988) was modified so as to be suitable for coupled transcription-translation, a process useful for obtaining products of **cloned** genes or cDNAs. A reaction chamber equipped with an ultrafiltration membrane was newly designed and an HPLC pump was used to supply a low-mol.-wt. substrate soln. at a const. rate to the viscous reaction mixt. in the chamber. By using an **Escherichia coli** S30 ext. in this modified flow system (1 mL), coupled transcription-translation could be continuously performed for 17 h, the **synthesized** chloramphenicol acetyltransferase (.simeq.0.1 mg) being subsequently eluted through the chamber membrane and then purified.

ST reactor continuous **protein synthesis** system; coupled transcription translation Escherichia; **cell free** system transcription translation

IT **Escherichia coli**
(continuous **cell-free protein synthesis** system for coupled translation and transcription based on exts. on)
IT **Protein formation**
(continuous **cell-free** system for, for coupled transcription-translation)
IT Deoxyribonucleic acids
RL: BIOL (Biological study)
(transcription of, translation coupled with, continuous **cell-free protein synthesis** system for)
IT Ribonucleic acids, messenger
RL: BIOL (Biological study)
(translation of, transcription coupled with, continuous **cell-free protein synthesis** system for)
IT Reactors
(ultrafiltration membrane combined with, in continuous **cell-free protein synthesis** system for coupled transcription-translation)
IT Filtering materials
(ultra-, membranes, in reaction chamber, for continuous **cell-free protein synthesis** system for coupled transcription-translation)
IT 9040-07-7P, Chloramphenicol acetyltransferase
RL: PREP (Preparation)
(prepn. of, in continuous **cell-free protein synthesis** system)

L60 ANSWER 50 OF 50 HCAPLUS COPYRIGHT 2003 ACS on STN
AN 1991:178686 HCAPLUS
DN 114:178686
TI Insulin and IGF-I induce pronounced hypertrophy of skeletal myofibers in tissue culture
AU Vandenburgh, Herman H.; Karlisch, Patricia; Shansky, Janet; Feldstein, Rebecca
CS Dep. Pathol., Brown Univ., Providence, RI, 02906, USA
SO American Journal of Physiology (1991), 260(3, Pt. 1), C475-C484
CODEN: AJPHAP; ISSN: 0002-9513
DT Journal
LA English
CC 2-6 (Mammalian Hormones)
AB Skeletal myofibers differentiated from primary avian myoblasts in tissue culture can be maintained in pos. N balance in a defined serum-**free** medium for at least 6-7 days when embedded in a **3-dimensional** collagen gel matrix. Incubation of established myofiber cultures for 3-7 days with insulin (1 .mu.M) or insulin-like growth factor I (IGF-I, 32 nM) stimulates both **cell** hyperplasia and myofiber hypertrophy. Mean myofiber diam. increases 71-98%. Insulin-like growth factor II stimulates **cell** hyperplasia but not myofiber hypertrophy. **Cell** growth results from a 42-62% increase in total **protein synthesis** and a 28-38% decrease in **protein** degrdn. Myosin heavy-chain content increases 183-258% because of a 55% stimulation of myosin **synthesis** and 33-61% inhibition of degrdn. Assocd. with myofiber hypertrophy is a 87-148% increase in the no. of myofiber nuclei per unit myofiber length. The results indicate that insulin and IGF-I, but not IGF-II, can induce rapid myofiber hypertrophy in vitro, most likely by stimulating myoblast proliferation and/or **fusion** to established myofibers.
ST insulin skeletal myofiber hypertrophy; insulin growth factor myofiber hypertrophy
IT Myosins
RL: BIOL (Biological study)

(heavy chain, formation of, by muscle myofibers in culture, insulin and insulin-like growth factor I stimulation of)

IT **Proteins**, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(metab. of, by muscle myofibers in culture, insulin and insulin-like growth factor I inhibition of)

IT **Protein formation**
(muscle cell growth stimulation by insulin and insulin-like growth factor I mediation by, in culture)

IT Muscle
(fiber, hypertrophy of, in culture, insulin and insulin-like growth factor I induction of)

IT 67763-97-7, Insulin-like growth factor II
RL: PROC (Process)
(muscle myofiber cell hyperplasia stimulation of, in culture)

IT 9004-10-8, Insulin, biological studies 67763-96-6, Insulin-like growth factor I
RL: BIOL (Biological study)
(muscle myofiber hypertrophy induction by, in culture)